# JOURNAL OF DAIRY SCIENCE

RECEIVED JUN 30 1945

## Contents

*Studies of the Carbohydrate Metabolism of Mammary Gland Tissue in Vitro. I. Production and Utilization of Various Carbohydrate Substances.* C. B. KNODT and W. E. PETERSEN ..... 415

*Negative Pressure and Nursing by Calves.* VEARL R. SMITH and W. E. PETERSEN ..... 431

*The Varying Requirement for Choline for Normal Metabolism of Proteins (Casein, Lactalbumin, and Soy Protein) with Particular Reference to the Hepatico-Renal Syndrome.* G. C. SUPPLEE, LORRAINE S. GALL and JEAN F. CAUL ..... 435

*A Comparison of Choline, Betaine and Dimethylaminoethanol in the Prevention of the Hepatico-Renal Syndrome.* G. C. SUPPLEE, JEAN F. CAUL, LORRAINE S. GALL and E. A. PRILL 455

*The Use of Simplified Diets in the Study of the Fat Metabolism of the Mammary Gland.* O. W. KAUFMANN and J. C. SHAW ..... 467

*The Vitamin A Potency of Creamery Butter Produced in Minnesota.* ROBERT JENNESS and L. S. PALMER ..... 473

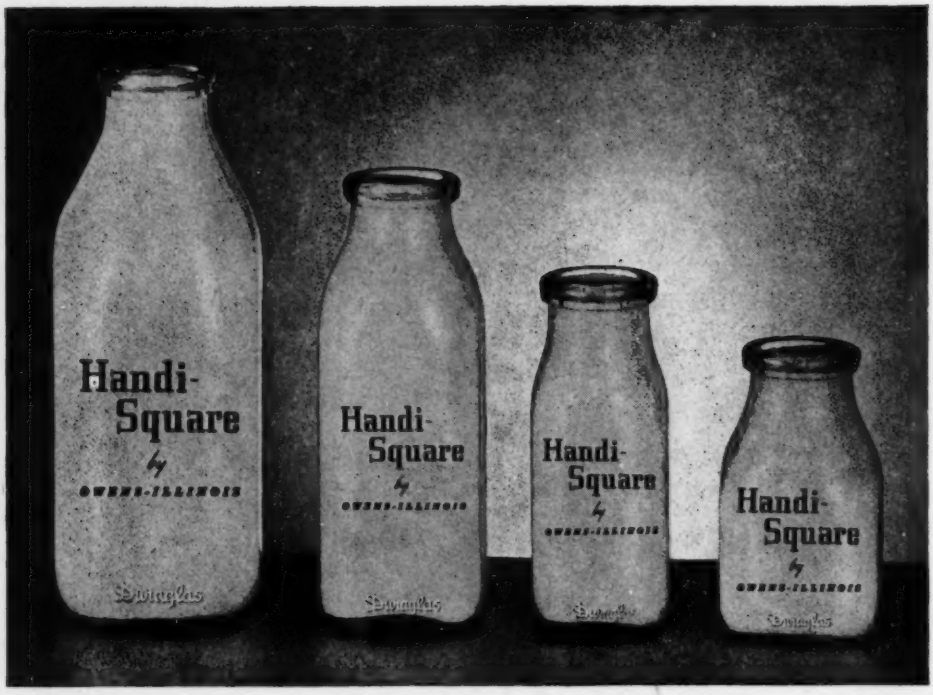
*The Relationship Between a Low Carotene Intake and Urinary Excretion of Ascorbic Acid in Dairy Cattle.* L. A. MOORE and J. W. COTTER ..... 495

*Note from the Editor* ..... 507

*Abstracts of Literature* ..... A75

Vol. XXVIII, No. 6, June, 1945

Published by the
AMERICAN DAIRY SCIENCE ASSOCIATION




# THE MARKETING OF MILK MOVES FORWARD

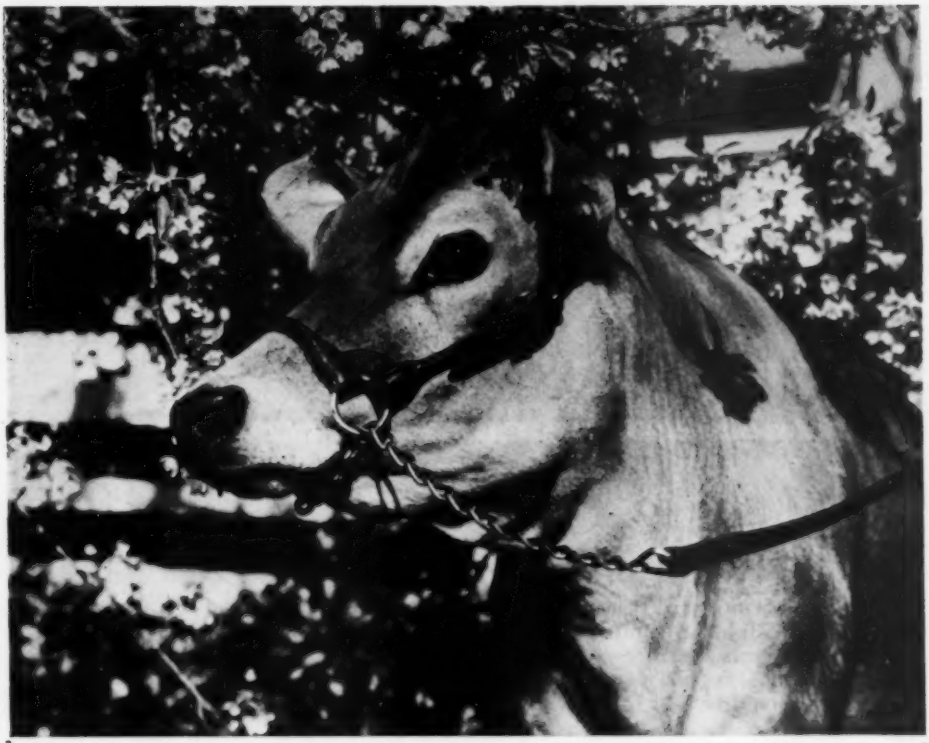
● Pacing developments in other areas of dairy development, the new Handi-Square Milk Bottles represent a long step ahead in the efficient marketing of milk.

To dairies and their customers, these scientifically designed containers—provide space saving in cold rooms, empty bottle storage, in delivery trucks, for you can put *three* cases of Handi-Squares in the same area as *two* of ordinary round bottles. They weigh 20% less than standard quarts—are easy to grip, to handle, to pour from. They don't roll if knocked over—are a better fit in a refrigerator.

These, and other improvements, feature the new Duraglas Handi-Squares. If you would like to know more about them, write for our booklet, "Handi-Square Milk Bottles" by Owens-Illinois.

**OWENS-ILLINOIS GLASS COMPANY**
**Dairy Container Division**
**TOLEDO 1, OHIO**

Your advertisement is being read in every State and in 35 Foreign Countries



**JERSEYS MATURE EARLY!**

**(Early Maturity Pays)**

**WELCOME VOLUNTEER 399447**

Has become the youngest Medal of Merit bull of the Jersey Breed.
His first 8 daughters average 715 lbs. fat on the 2x-305 day m.e. basis.
His three Medal of Merit daughters: (Actual Records)

Welcome Volunteer Sable 1305780 (Very Good)
3–2—305—14,729 lbs. milk, 6.35%, 935 lbs. fat

Welcome Volunteer Foxy 1305779 (Good Plus)
3–2—305—13,554 lbs. milk, 5.79%, 779 lbs. fat

Welcome Volunteer Jiff 1316589 (Good Plus)
2–11—305—13,738 lbs. milk, 5.48%, 752 lbs. fat

This great sire was developed by Orrland Farm, Tulia, Texas (down in the Panhandle country).

Another little breeder attains to the highest honor following a project of the Jersey Breed.

**The American Jersey Cattle Club**

**324 West 23rd Street**

**NEW YORK 11, NEW YORK**

**Your advertisement is being read in every State and in 25 Foreign Countries**

# JOURNAL OF DAIRY SCIENCE

OFFICIAL ORGAN OF
AMERICAN DAIRY SCIENCE ASSOCIATION

Published at
NORTH QUEEN ST. AND MCGOVERN AVE., LANCASTER, PA.



T. S. SUTTON, Editor
Columbus, Ohio

*Associate Editors*

| | | | |
|---|---|---|---|
| O. F. HUNZIKER<br>La Grange, Ill. | C. A. CARY<br>Beltsville, Md. | I. W. RUPEL<br>Madison, Wis. | H. A. RUEHE<br>Chicago, Ill. |
| I. A. GOULD<br>East Lansing, Mich. | E. G. HASTINGS<br>Madison, Wis. | PAUL F. SHARP<br>Piedmont, Calif. | P. R. ELLIKER<br>Lafayette, Ind. |

*Committee on Journal Management*
FORDYCE ELY, *Chairman*

G. E. HOLM
T. S. SUTTON, *ex officio*
G. M. TROUT
R. B. STOLTZ, *ex officio*


---


*Subscriptions.* Price; $6.00 per volume in North and South America; $6.50 in all other countries. Prices are net, postpaid. New subscriptions and renewals are entered to begin with the first issue of the current volume. Renewals should be made promptly to avoid a break in the series. Subscriptions should be sent to R. B. Stoltz, The Ohio State University, Columbus, Ohio.

Subscriptions for the British Isles and British Empire, except for Canada and Australia, should be ordered through our agents: Messrs. Bailliere, Tindall and Cox, 7 and 8 Henrietta Streets, Covent Garden, London, W. C. 2, England. Subscriptions for Australia should be sent to our agent: John H. Bryant, Herbert St., St. Leonards, N. S. W., Australia.

*Advertising* should be mailed direct to the Science Press Printing Company, N. Queen St. and McGovern Ave., Lancaster, Pennsylvania.

*Post Office Notices* of undeliverable copies and changes of address should be sent to R. B. Stoltz, The Ohio State University, Columbus, Ohio.



OFFICERS OF THE ASSOCIATION

A. C. RAGSDALE, President
Columbia, Missouri

J. A. NELSON, Vice-President
Bozeman, Montana

R. B. STOLTZ, Sec.-Treas.
Columbus, Ohio

DIRECTORS

| | | |
|---|---|---|
| P. H. TRACY<br>Urbana, Illinois | W. E. PETERSEN<br>St. Paul, Minn. | P. A. DOWNS<br>Lincoln, Nebr. |
| C. L. BLACKMAN<br>Columbus, Ohio | R. B. BECKER<br>Gainesville, Fla. | J. W. LINN<br>Manhattan, Kans. |
| | A. C. DAHLBERG<br>Ithaca, New York | |


---


Entered as second-class matter April 13, 1934 at the postoffice at Lancaster, Pa., under the act of March 3, 1879.

"IT WON'T BE LONG NOW!"

He's right! With V-E Day past, hopes of better delivery are brighter. Although we cannot make any definite promises for the immediate future, it is reasonable to assume that more manpower and material should soon be available. Of course, with our large backlog of orders it will still be wise to place your orders for equipment early.

The present priority system will, no doubt, continue for some time so it will still be mandatory that we deliver on a first come, first served basis.

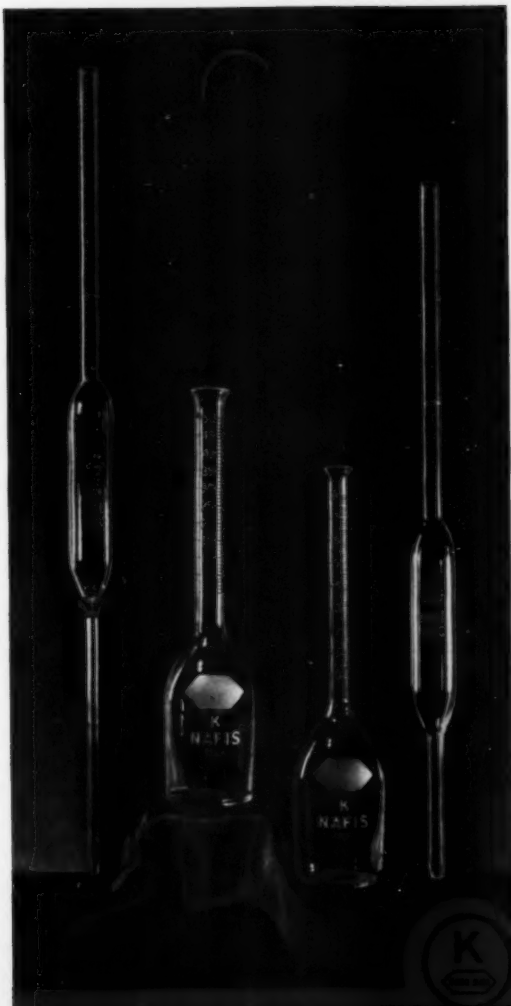
ESSENTIAL DAIRY FOODS

**GENERAL DAIRY EQUIPMENT CO., Minneapolis 14, Minn.**

# KIMBLE K T.M. REG. U.S.A. BRAND

*For Assurance*

## DAIRY GLASSWARE




*Retested*
*Retempered*

**Colored Fused-in Glass filler in lines and numbers**

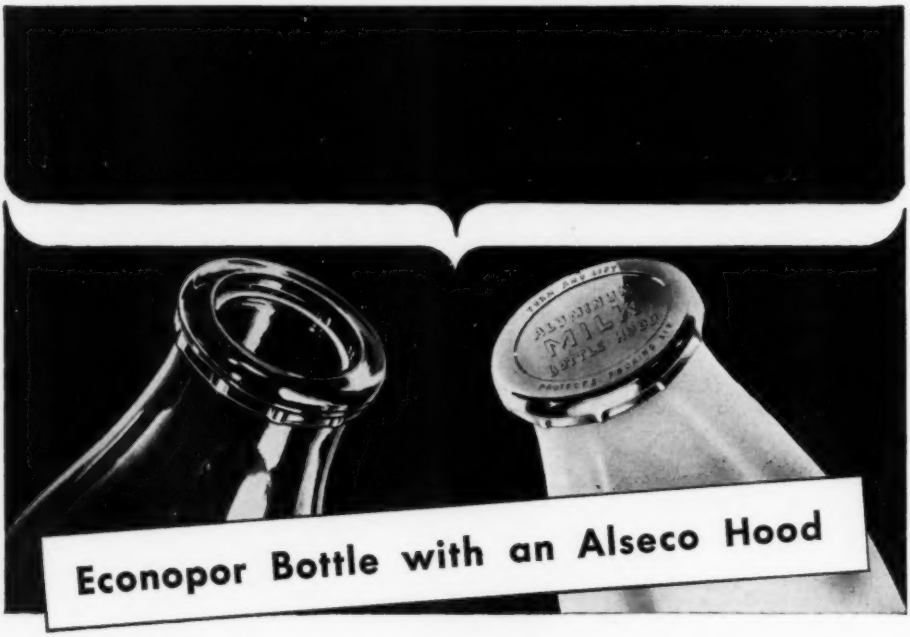
Kimble Babcock Bottles and Pipettes meet all requirements of the National Bureau of Standards, the American Dairy Science Association, the Association of Official Agricultural Chemists, and the regulations of the various States.

Distributed by Leading Dairy Supply Houses Throughout the United States and Canada

*The Visible Guarantee of Invisible Quality*

KIMBLE GLASS COMPANY · · · · VINELAND, N. J.

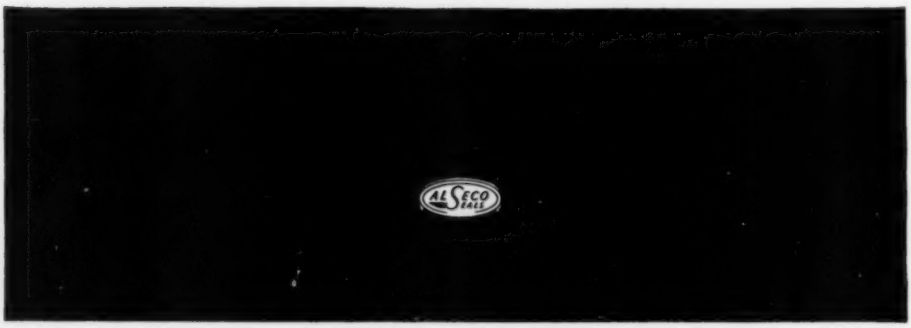
Your advertisement is being read in every State and in 25 Foreign Countries




By standardizing now on the Econopor Bottle with lugs, which accommodates any type of cap or hood, a dairy can be ready to adopt Alseco Aluminum Hoods when they are again available. Alseco Hoods have these nine advantages:

1. They cover the pouring lip; keep it clean.
2. They seal the bottle; no plug cap needed.
3. They are tamperproof, odor-proof, tasteproof.
4. They have more eye-appeal; help sell milk.
5. They withstand ordinary methods used in icing cases.
6. They are easy for women to remove and replace.
7. They are cheaply applied by automatic machines.
8. They are approved by health authorities.
9. They are economical for any size dairy.

Now is the time to lay plans for changing to Alseco Hoods. For information on how to go about it, write ALUMINUM SEAL COMPANY, 1315 Third Avenue, New Kensington, Pa.




Your advertisement is being read in every State and in 25 Foreign Countries

# Never a dull moment

As a good dairyman who shudders at stains and dull tarnish on dairy equipment, a lasting treat awaits you—in Wyandotte! And meant especially for you is *this* Wyandotte threesome—C.W., G.L.X.,* and Steri-Chlor.*

For washing cans by machine, C.W. is amazingly thorough. Non-corroding, leaving no discolorations, it takes hard water in stride. And C.W. erases foreign substances *right now,* and at low cost—to leave tin and other metal surfaces bright and *sanitary.*

When G.L.X. is bowed in, deposits on surfaces of monel, steel and glass bow *out*—pronto! A "sudsing" cleaner without soap, G.L.X. goes to town on casein-coated equipment.

* Registered trademark.

And for that final germicidal rinse *after* cleaning—comes Steri-Chlor. Spelling instant doom for lingering bacteria, Steri-Chlor is swift, sure, *safe.*

**FOR YOUR DAIRY FARMERS**

Here are some of the Wyandotte Products needed by farmers—and packaged conveniently for their use:

- **G. L. X.**—Sudsy, all-round dairy utensil cleaner.
- **Wyandotte Cleaner and Cleanser**—Non-sudsy, for soft or medium hard water.
- **Wyandotte Steri-Chlor**—Destroys bacteria and odors *safely.*

Wyandotte
REG. U. S. PAT. OFF.

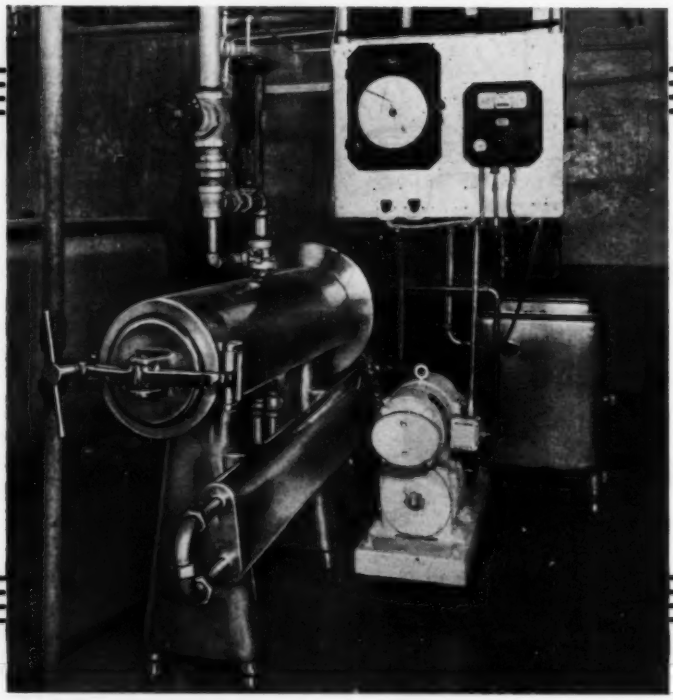
*SERVICE REPRESENTATIVES IN 88 CITIES*

**WYANDOTTE CHEMICALS CORPORATION • J. B. FORD DIVISION • WYANDOTTE, MICHIGAN**

**Your advertisement is being read in every State and in 25 Foreign Countries**

*natural flavor . . .*
*low bacteria count*

**at VON ALLMEN BROS. DAIRY, Louisville, Ky.**



"The high quality product we are producing with the Mojonnier 16-Second Pasteurizing System is the largest contributing factor in our growth, which more than doubled during the first year of our operation," said Mr. Julius Von Allmen, President.

Handling 5000 lbs. of milk per hour, practically all Von Allmen products are run through this system.

*Write for full details today to:*

**MOJONNIER BROS. CO.**
4601 W. OHIO STREET • CHICAGO 44, ILLINOIS

**Mojonnier**

**SHORT-TIME PASTEURIZING SYSTEM**

**Your advertisement is being read in every State and in 25 Foreign Countries**

# JOURNAL OF DAIRY SCIENCE

VOLUME XXVIII JUNE, 1945 NUMBER 6

## STUDIES OF THE CARBOHYDRATE METABOLISM OF MAMMARY GLAND TISSUE IN VITRO. I. PRODUCTION AND UTILIZATION OF VARIOUS CARBOHYDRATE SUBSTANCES[1]

C. B. KNODT AND W. E. PETERSEN

*Dairy Division, University of Minnesota*

The problem of lactose formation in the mammary gland has been attacked by a number of different methods largely with a view of establishing the blood precursors. Production of hypoglycemia by inanition (13, 33) and by insulin and phloridzin administration (6, 9, 14, 22, 27, 32, 34, 35) resulted in lowered lactose content of the milk. Hyperglycemia has been produced by intravascular infusion of glucose (7, 31), introduction of large amounts of glucose into the rumen (24), infusion of sugars into the udder (8), injection of thyroxine (25), and by implantation of epinephrine tablets (5). The effects of the removal of the mammary glands just prior to parturition (3, 28, 30) appear to be somewhat questionable.

Further attempts at establishing the probable blood precursors of lactose have been made by determining the arterio-venous difference for various blood constituents. It is well established that large amounts of glucose are taken up by the mammary glands (4, 26, 16, 17, 18, 47). The utilization of lactic acid is reported by some (15, 47) while others (40) could not confirm these reports in non-excited cows. Removal of the carbohydrate portion of the glycoproteins from the traversing blood has been reported (41). Perfusion of the excised mammary glands (39) and the use of tissue slices (19, 20) and breis (2, 23, 29, 37, 43, 49) have been employed to study the precursors of lactose.

While much work has been done on the probable blood precursors of milk sugar, there still remains a number of blood constituents to be investigated. Among these are citric acid, pyruvic acid, and $\alpha$-keto glutaric acid. Also unsolved is the metabolism relating to the synthesis of lactose in the mammary gland. The only attempts to study this phase of the problem has been by the use of tissue slices and breis.

Received for publication December 1, 1944.

[1] Taken from data presented in a thesis to graduate faculty of the University of Minnesota by C. B. Knodt in partial fulfillment of the requirements for the degree of Doctor of Philosophy. Scientific Journal Series Paper No. 2207. Minnesota Agricultural Experiment Station.



Copyright, 1945, by the AMERICAN DAIRY SCIENCE ASSOCIATION.

Petersen and Shaw (37) demonstrated lactose formation from glucose and lactic acid in breis and Grant (19, 20) from glucose additions to tissue slices. Grant could not demonstrate lactose formation from such carbohydrate metabolism intermediates as hexosephosphates and phosphoglyceric acid additions to tissue breis. To add to the knowledge of the carbohydrate metabolism in the mammary gland, a series of experiments was set up using isolated tissues and the perfusion techniques.

## EXPERIMENTAL PROCEDURE

In all of the experiments, bovine mammary glands obtained from two local abattoirs were used. Special attention was given to the selection, with a view of obtaining glands in the early stages of lactation producing apparently normal milk. The glands were removed from the animal within 15 to 20 minutes after slaughter and taken to the laboratory. The experiments were in progress within 1 to 1.5 hours post mortem. Some of the glands were perfused by modifications of the Petersen *et al.* technique (39) while the tissue of others was incubated as breis and slices. In the perfusion experiments, chlorazol fast pink was used as the anticoagulant. The tissue breis were prepared by use of the regular household type meat grinder. The tissue slices were made by use of the standard high-speed meat slicer. The average thickness of these slices was less than 2 mm., and in the later stages of this work they were usually less than 1 mm. in thickness. All incubations were conducted at 37.5° C. There were many variations in the treatment of the various experiments involving perfusion and incubation of breis and slices, the details of which are best considered under the presentation and discussion of the data for the several types of experiments.

The methods for the determination of lactose in the presence of other sugars are all, at present, subject to certain criticisms. Because of the relative ease of manipulation and accuracy under the conditions of these experiments, the method of Scott and West (45) was used for the determination of a fraction which is referred to as lactose. Maltose was used in some of the experiments but, by the use of the method of Schultz and Akin (44), was rapidly fermented off and did not interfere with the analysis for lactose. All large pieces of tissue were finely ground as in the case of those for the determinations in table 3. In the case of breis and slices and the above, the tissue was washed with a total of 10 times its weight of distilled water, with vigorous shaking and centrifuging between each of 5 washings.

The following chemical techniques were also used; glucose, Shaffer and Somogyi (46); lactic acid, Barker and Summerson (1); pyruvic acid, Friedemann and Haugen (11); glycogen, Good, Kramer, and Somogyi (12). The glycogen used was prepared according to the method of Somogyi (48), from calf fetal livers.

## EXPERIMENTAL RESULTS

### IN VITRO GLYCOGEN METABOLISM

A series of 20 experiments, as presented in table 1, was conducted to determine if the very low glycogen level of udders transported from the abattoir could be increased by perfusion with high concentrations of blood glucose. The udders were split medially, and one half was analyzed immediately upon its arrival in the laboratory while the other half was perfused for 4 to 5 hours. It will be observed that the average glycogen content of the tissue before perfusion was only 25.11 mg. per cent. This low concentration was due to the glycogen catabolism which occurred during the transportation of the gland from the abattoir. The perfused half glands average 231.7 mg. per cent glycogen following perfusion, or an average increase of 206.6 mg. per cent in the concentration of glycogen in the tissue.

TABLE 1

*The effect of the perfusion of excised mammary glands with glucose upon tissue glycogen concentration*

| Perfusion number | Tissue glycogen (mg. %) | | |
|---|---|---|---|
| | Before perfusion | After perfusion | Difference |
| 155 | 21.8 | 93.2 | 71.4 |
| 156 | 27.9 | 126.4 | 98.5 |
| 157 | 21.9 | 273.7 | 251.8 |
| 158 | 28.2 | 132.0 | 103.8 |
| 159 | 23.8 | 256.4 | 232.6 |
| 160 | 14.0 | 336.1 | 322.1 |
| 161 | 29.5 | 162.5 | 133.0 |
| 162 | 22.8 | 27.2 | 4.4 |
| 163 | 21.3 | 31.7 | 10.4 |
| 164 | 13.4 | 123.4 | 110.0 |
| 166 | 28.0 | 275.0 | 247.0 |
| 167 | 27.4 | 42.8 | 15.4 |
| 168 | 22.6 | 246.5 | 223.9 |
| 169 | 36.6 | 429.6 | 393.0 |
| 170 | 20.8 | 428.1 | 407.3 |
| 171 | 30.3 | 194.3 | 164.0 |
| 172 | 29.6 | 245.9 | 216.3 |
| 174 | 26.4 | 392.0 | 365.6 |
| 175 | 34.3 | 514.2 | 479.9 |
| 181 | 21.5 | 302.9 | 281.4 |
| Average (20 expts.) | 25.11 | 231.7 | 206.6 |

While there is great variation in the mammary gland glycogen content in these experiments, there are significant increases in all but perhaps three cases. The small increases in perfusions 162, 163 and 167 are attributable to a marked and unexplainable vasoconstriction encountered in these experiments. It is of special interest that, by perfusion with high concentrations of blood glucose, the mammary gland glycogen can be built up to levels comparable to the 200 mg. per cent reported for normal lactating glands (38).

The highest concentration obtained was 514.2 mg. per cent, or 2.5 times the normal average.

Since tissue glycogen could be increased by perfusion with blood containing high concentrations of glucose, attempts were also made to increase tissue glycogen in mammary tissue breis by the use of various compounds, but were all unsuccessful. In these experiments 25 cc. of a 0.9 per cent sodium chloride solution containing 1 per cent of the various compounds and combinations was tried; namely, glucose, lactic acid, citric acid, and pyruvic acid and were incubated for 6 hours. One hundred mg. of glycogen was also added to some of these incubations, but the results were all negative. It was added because of the effects of added glycogen upon the formation of this substance by phosphorylase, as shown by Green *et al.* (21) and Cori and Cori (10). Insulin was added to observe whether or not glycogen might accumulate, but this was also unsuccessful in incubations of tissue breis.

These experiments demonstrate that glycogen can be formed in the perfused mammary gland and that, by use of large amounts of added glucose, a concentration can be reached which is comparable to that of the intact gland. Whether the glycogen serves merely as a reservoir built up during periods of surplus blood glucose and drawn upon during periods of stress or as an intermediary through which all carbohydrates used by the gland must pass is, of course, not revealed by this work.

### CONVERSION OF GLYCOGEN AND OTHER SUBSTANCES TO LACTOSE

Since it was found possible to increase the concentration of glycogen by perfusing lactating mammary glands, experiments were conducted to determine if glycogen so formed could be converted to lactose during the incubation of the perfused tissue. The udders were divided medially, and one half of the udder was perfused with high concentrations of glucose ranging up to 5,611 mg. per cent while the other half served as a control and was incubated but not perfused. A portion of about 200 gm. of unperfused tissue was incubated at 37.5° C. for 6 hours. The perfused tissue was also incubated for 6 hours following perfusion. The results of these experiments are presented in table 2.

In the nonperfused portion there was an average of 26.13 mg. per cent tissue glycogen before incubation, which decreased to an average of 13.34 mg. per cent during incubation. No fermentable reducing sugar was detected either before or after incubation. The lactose content decreased from an average of 486.2 mg. per cent at the beginning to 440.3 mg. per cent at the end, or an average loss of 45.9 mg. per cent.

In the perfused portion the glycogen content averaged 329.2 mg. per cent at the beginning, which dropped to an average of 45.70 mg. per cent at the end of the incubation, or a loss of 283.5 mg. per cent. At the beginning of incubation the tissue contained an average of 103.3 mg. per cent fermentable

TABLE 2

*The metabolism of lactose* in incubated perfused and unperfused mammary gland tissue*

| Perfusion number | Unperfused half of udder | | | | | | Perfused half of udder | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Glycogen | | Fermentable sugar | | Lactose | | Glycogen | | Fermentable sugar | | Lactose | |
| | Before incub. | After incub. | Before incub. | After incub. | Before incub. | After incub. | Before incub. | After incub. | Before incub. | After incub. | Before incub. | After incub. |
| 159 | 23.82 | 21.60 | 0.0 | 0.0 | 550.1 | 429.6 | 256.4 | 31.40 | 112.0 | 0.0 | 637.2 | 792.5 |
| 160 | 13.98 | 9.16 | 0.0 | 0.0 | 572.1 | 467.6 | 336.1 | 27.60 | 72.00 | 0.0 | 772.1 | 903.8 |
| 166 | 28.04 | 15.07 | 0.0 | 0.0 | 329.1 | 411.3 | 275.0 | 87.12 | 91.34 | 0.0 | 411.3 | 529.9 |
| 168 | 22.64 | 16.20 | 0.0 | 0.0 | 467.0 | 408.5 | 246.5 | 36.42 | 80.52 | 0.0 | 521.6 | 692.1 |
| 169 | 36.22 | 18.86 | 0.0 | 0.0 | 563.0 | 490.6 | 429.6 | 56.01 | 103.3 | 0.0 | 614.1 | 788.3 |
| 170 | 20.76 | 10.79 | 0.0 | 0.0 | 521.7 | 485.6 | 428.1 | 51.30 | 173.6 | 0.0 | 602.9 | 813.8 |
| 171 | 30.27 | 11.26 | 0.0 | 0.0 | 379.3 | 351.8 | 194.3 | 25.80 | 67.21 | 0.0 | 441.7 | 509.5 |
| 172 | 29.60 | 9.26 | 0.0 | 0.0 | 513.2 | 468.3 | 245.9 | 17.92 | 64.25 | 0.0 | 607.4 | 715.9 |
| 174 | 26.41 | 6.45 | 0.0 | 0.0 | 574.0 | 552.3 | 392.0 | 47.29 | 74.28 | 0.0 | 622.3 | 791.2 |
| 175 | 34.25 | 9.18 | 0.0 | 0.0 | 477.0 | 403.7 | 514.2 | 76.24 | 139.4 | 0.0 | 522.1 | 792.5 |
| 181 | 21.47 | 12.81 | 0.0 | 0.0 | 401.9 | 374.6 | 302.9 | 45.62 | 158.5 | 0.0 | 483.8 | 598.1 |
| Average ..... | 26.13 | 12.79 | 0.0 | 0.0 | 486.2 | 440.3 | 329.2 | 45.70 | 103.3 | 0.0 | 567.0 | 720.9 |
| Average Diff. | 1 | 13.34 | 0.0 | 0.0 | ..... | −45.9 | ..... | 283.5 | ..... | 103.3 | ..... | 153.9 |

* Expressed as mg. % in tissue.

sugar, presumably glucose. All of the fermentable sugar disappeared during incubation. The average lactose content increased from 567.0 mg. per cent at the beginning to 720.9 mg. per cent at the end of incubation.

To overcome the effect of the presence of large amounts of glucose in the perfused glands which were incubated, mammary gland tissue breis and slices were incubated in glycogen solutions. To 25 gm. of tissue brei was added 200 mg. of glycogen and incubation carried on for 6 hours. The results of these experiments are presented for breis in table 3. It will be noted that there was the expected disappearance of glycogen, but lactose was not formed.

A series of experiments was conducted to study lactose formation in mammary tissue breis and slices from the addition of various possible lactose precursors. The substances studied were: glucose, lactic acid, citric acid,

TABLE 3

*The relation of glycogen breakdown to lactose formation in tissue breis. (Incubated for 6 hours at 37.5° C.)*

| Experiments number | Lactose (mg. present) | | Glycogen (mg. present) | |
|---|---|---|---|---|
| | Before incub. | After incub. | Before incub. | After incub. |
| 1 | 136.7 | 133.2 | * | 46.0 |
| 2 | 121.4 | 118.2 | * | 71.5 |
| 3 | 123.8 | 125.6 | * | 37.2 |
| 4 | 129.4 | 126.3 | * | 42.9 |
| 5 | 125.0 | 128.8 | * | 54.7 |
| Average ..... | 127.3 | 126.3 | * | 50.5 |

* 200 mg. of glycogen added prior to incubation with 25 gm. of macerated mammary gland tissue.

pyruvic acid, glycogen and maltose. In all experiments, 25 gm. of tissue and 25 cc. of isotonic fluid were used.

Most of the work was done on tissue slices, as a series of experiments in which various substances were added to breis failed to synthesize appreciable amounts of lactose. The data obtained upon 3 groups of experiments in which the addition of glucose, glucose and lactic acid, glycogen, and glycogen and lactic acid were made to tissue breis are presented in table 4. Although the additions prevented the degradation of lactose as contrasted to the losses observed in the control, there is no evidence that lactose synthesis occurred. Apparently the tissue cells must have been too severely damaged in the production of the brei to function normally.

In the study of lactose formation with the use of tissue slices, the saline solution used was that of Robison and Rosenheim (42), containing 10 mg. calcium and 3 mg. inorganic phosphate in addition to 6.0 gm. of sodium chloride, 2.2 gm. sodium bicarbonate, 0.3 gm. potassium chloride, 0.25 gm. magnesium sulphate per liter. This was then mixed with osmotically iso-

tonic solutions of other compounds used to give the desired concentrations of the compound involved, with resulting total of 25 cc. of fluid. Glycogen was made up in a 1 per cent solution and used directly, with sufficient potassium hydroxide and hydrochloric acid being used to give the solution the proper osmotic pressure and pH.

The results of six experiments run in duplicate, in which glucose, glucose and lactic acid, lactic acid, citric acid, pyruvic acid, maltose, and glycogen were added to tissue slices to make a one per cent concentration of the substrate, are given in table 5. At the beginning the tissue contained an average of 518.9 mg. per cent lactose. An average loss of 64.6 mg. per cent of lactose was observed in the controls. There was an average formation of

TABLE 4

*Effect of various substances upon lactose* metabolism in tissue breis*

| | Experiments | | | Average |
|---|---|---|---|---|
| | 1 | 2 | 3 | |
| Before incubation Control | 537.5 | 504.2 | 493.8 | 511.8 |
| After incubation Control | 483.0 | 462.7 | 459.8 | 468.5 |
| 5.34% glucose | 521.6 | 493.8 | 496.1 | 503.8 |
| 2.67% glucose and 1.34% lactic acid | 516.8 | 486.5 | 503.0 | 502.1 |
| 1% glycogen | 527.4 | 502.7 | 505.0 | 511.7 |
| 1% glycogen and 1.34% lactic acid | 520.8 | 511.9 | 489.4 | 507.4 |
| 1% glycogen and 2.68% lactic acid | 531.6 | 503.2 | 491.4 | 508.7 |

* Expressed as mg. % present in the tissue.

140.6 mg. per cent lactose during the incubations with added glucose; and the experiments with added glucose and lactic acid, maltose and glycogen are not significantly different. Examination of the data in table 5, however, shows considerable variation in the amounts of lactose produced, with a variation of as much as 59.5 mg. per cent between formation from these different compounds.

The addition of lactic acid, pyruvic acid, and citric acid failed to increase the lactose concentration, but did prevent to some extent the degradation of lactose. There was again considerable variation in this respect between different experiments and also between the observed effect of these compounds in a given set of incubations.

The observations relative to lactic acid metabolism are presented in table 6. Incubation of the controls brought about an increase of 10.60 mg. per cent lactic acid. Additions of glucose, citric acid, pyruvic acid, maltose and glycogen produced higher final concentrations of lactic acid. The mag-

TABLE 5

*The metabolism of lactose* during incubation of mammary gland tissue slices*

| Experiment number | Before incubation | After incubation | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Control | Control | 1% Glucose | 0.5% glucose & 0.5% lactic acid | 1% lactic acid | 1% citric acid | 1% pyruvic acid | 1% maltose | 1% glycogen |
| 1 | 523.4 | 416.8 | 708.7 | 689.6 | 499.2 | 505.3 | 503.4 | 711.9 | 740.7 |
| 2 | 477.0 | 419.3 | 574.5 | 581.2 | 459.3 | 445.7 | 463.5 | 589.4 | 597.6 |
| 3 | 508.2 | 412.9 | 639.3 | 620.6 | 487.8 | 504.3 | 495.0 | 664.7 | 680.1 |
| 4 | 575.5 | 544.3 | 739.2 | 700.6 | 499.0 | 540.9 | 521.4 | 721.5 | 742.6 |
| 5 | 529.4 | 489.0 | 662.7 | 629.5 | 527.8 | 509.4 | 520.0 | 630.7 | 608.1 |
| 6 | 499.9 | 443.7 | 635.0 | 647.2 | 478.3 | 463.1 | 495.3 | 648.5 | 652.0 |
| Average ..... | 518.9 | 454.3 | 659.5 | 644.8 | 491.9 | 494.8 | 499.8 | 661.1 | 670.2 |

* Expressed as mg. % present in the tissue.

TABLE 6

*The metabolism of lactic acid* during incubation of mammary gland tissue slices*

| Experiment number | Before incubation | | | After incubation | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control | Control & 1% lactic acid | Control & 0.5% lactic acid | Control | 1% glucose | 0.5% glucose & 0.5% lactic acid | 1% lactic acid | 1% citric acid | 1% pyruvic acid | 1% maltose | 1% glycogen |
| 1 | 22.40 | 1028.5 | 520.0 | 39.91 | 84.33 | 484.8 | 990.0 | 53.34 | 70.08 | 68.18 | 74.18 |
| 2 | 20.02 | 1022.7 | 514.0 | 32.32 | 74.80 | 512.2 | 1005.3 | 37.75 | 70.13 | 69.47 | 55.82 |
| 3 | 20.62 | 1009.7 | 514.4 | 31.72 | 82.16 | 495.3 | 992.5 | 46.02 | 74.30 | 70.15 | 60.22 |
| 4 | 30.41 | 1010.3 | 526.8 | 41.76 | 82.90 | 502.6 | 1011.9 | 57.21 | 78.69 | 74.05 | 67.38 |
| 5 | 23.00 | 1021.8 | 515.1 | 35.10 | 81.36 | 479.4 | 995.0 | 41.33 | 71.42 | 76.00 | 58.37 |
| 6 | 26.58 | 1005.0 | 509.9 | 25.75 | 71.43 | 486.6 | 967.2 | 31.75 | 67.51 | 53.36 | 58.41 |
| Average ..... | 23.83 | 1016.3 | 516.7 | 34.43 | 79.50 | 493.5 | 985.3 | 44.58 | 72.02 | 68.53 | 62.40 |

* Expressed as mg. % in tissue.

nitude of the increases is not significantly different for these substances, except for citric acid, which is somewhat lower. Where lactic acid was added in large amounts, there is some evidence of a small utilization of this substance.

No evidence has been presented to show that mammary gland tissue utilizes pyruvic acid; consequently, it was deemed advisable to ascertain whether or not this substance is used by the active gland tissue. Pyruvic acid could not be detected in any incubations where it was not added. Six experiments were conducted in which 25 cc. of a 1 per cent solution of pyruvic acid was added to 25 gm. of tissue slices. The results of these experiments are presented in table 7. It will be noted that there is a marked

TABLE 7

*Utilization of added pyruvic acid by incubating mammary gland tissue*

| Incubation number | Concentration of pyruvic acid | |
|---|---|---|
| | Before incubation | After incubation |
| 1 | 1018.2 | 116.08 |
| 2 | 1008.0 | 245.30 |
| 3 | 1043.6 | 116.06 |
| 4 | 1009.4 | 204.40 |
| 5 | 1033.8 | 187.48 |
| 6 | 981.4 | 128.72 |
| Average (mg. % in fluid) | 1015.8 | 166.34 |
| Average (mg. present) | 253.9 | 41.58 |

decrease in pyruvic acid during incubation. Over a six-hour period, 25 gm. of tissue utilized 212.4 mg. of pyruvic acid out of 254 mg. present at the beginning of the incubation.

Since enzyme systems are usually sensitive to hydrogen ion concentrations, it was decided to ascertain the effect of different pH values that were thought to be important in lactose synthesis, namely, pH 6.6 to 7.2. In these experiments an isotonic solution of potassium phosphates with a pH of 7.4 was utilized and then adjusted to pH's of 6.6, 6.8, 7.0 and 7.2 by means of the addition of phosphoric acid. An isotonic glucose solution was then diluted with the phosphate saline to give a concentration of 1500 mg. per cent of glucose.

These data are presented in table 8. The pH of the incubating fluids did not change during incubation. There was a utilization of glucose and formation of lactic acid and lactose in all of these experiments where glucose was added. There was not a significant difference in the rate of lactose utilization and lactic acid formation which could be related to the differences in pH.

## DISCUSSION

It was possible to increase the glycogen level of excised mammary glands,

TABLE 8

*Effect of pH upon the formation of lactose and lactic acid by mammary gland tissue slices**

| Experiment numbers | Beginning | 3 hours incubation | | | | 6 hours incubation | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 6.6 | 6.8 | 7.0 | 7.2 | 6.6 | 6.8 | 7.0 | 7.2 |
| **Glucose present** | | | | | | | | | |
| 1 | 1575.0 | 986.4 | 1118.0 | 1088.1 | 1030.8 | 620.7 | 542.5 | 655.0 | 546.1 |
| 2 | 1493.0 | 1017.4 | 1092.3 | 986.4 | 1022.5 | 746.6 | 730.2 | 811.5 | 751.0 |
| 3 | 1520.0 | 1108.2 | 1034.0 | 1076.2 | 986.2 | 625.2 | 701.7 | 604.4 | 637.8 |
| Average | 1529.0 | 1037.3 | 1081.4 | 1050.2 | 1013.2 | 664.2 | 658.1 | 690.3 | 645.0 |
| **Lactose present** | | | | | | | | | |
| 1 | 507.9 | 597.3 | 597.3 | 588.0 | 597.3 | 642.0 | 628.0 | 652.0 | 661.0 |
| 2 | 586.2 | 671.2 | 694.8 | 660.5 | 678.4 | 716.5 | 722.9 | 714.3 | 707.3 |
| 3 | 562.2 | 607.0 | 624.1 | 595.2 | 608.0 | 674.1 | 660.4 | 685.5 | 657.2 |
| Average | 552.1 | 625.2 | 638.7 | 614.6 | 627.9 | 677.5 | 670.4 | 683.9 | 675.2 |
| **Lactic acid present** | | | | | | | | | |
| 1 | 28.73 | 75.70 | 79.65 | 92.23 | 79.88 | 115.9 | 127.8 | 151.5 | 120.0 |
| 2 | 13.53 | 76.43 | 72.81 | 83.70 | 68.33 | 93.71 | 102.0 | 95.84 | 88.92 |
| 3 | 12.10 | 96.28 | 74.39 | 70.15 | 81.26 | 94.28 | 92.16 | 103.7 | 86.52 |
| Average | 18.12 | 82.80 | 75.62 | 82.03 | 76.49 | 101.3 | 107.3 | 117.0 | 98.48 |

* Expressed as mg. % on basis of tissue.

which were very low in tissue glycogen following the transportation of them to the laboratory from the abattoir, by perfusion with large amounts of glucose to an average tissue glycogen concentration equal to and above that reported for normal intact mammary glands. This presents evidence of its formation in these excised glands and suggests its possible importance in mammary gland carbohydrate metabolism. It was also observed that the glycogen thus formed might be converted to lactose during the incubation of the perfused tissue. This did not necessarily prove that lactose is formed entirely from glycogen, but rather that glycogen might serve as a source of material for lactose formation.

In experiments involving the use of tissue breis, it was impossible to form lactose from glycogen or from glucose, lactic acid, pyruvic acid and citric acid individually or in various combinations. Lactose was formed by tissue slices when glycogen, glucose, glucose and lactic acid, and maltose were used as substrates.

The importance of lactic acid metabolism is still somewhat questionable, but it was found in incubation that this substance is formed from glucose, maltose, glycogen, pyruvic acid, and citric acid. This was also observed in the control incubations where no additions were made. Lactic acid utilization was observed where exceedingly high concentrations of lactic acid were added.

The utilization of pyruvic acid by mammary tissue has not previously been determined in any way. In a series of incubations in which glucose, lactic acid, citric acid, maltose, and glycogen were added, pyruvic acid was not determinable either before or after incubation. In experiments where large amounts of pyruvic acid were added, there was a considerable utilization of this compound. It was converted in part to lactic acid and citric acid. The importance of this substance in lactose formation is still undetermined.

It is generally accepted that each enzyme system has a certain pH for optimum activity. Incubations of mammary gland tissue slices and added glucose were maintained by use of four isotonic phosphate buffer solutions, with added glucose at a pH of 6.6, 6.8, 7.0 and 7.2 respectively. There was no significant difference in lactose formation, glucose utilization or lactic acid production in these experiments; and therefore it would appear that, within this range, pH is not a factor in lactose synthesis.

## SUMMARY

1. It has been possible to increase the concentration of gycogen in perfused mammary gland tissue to a level comparable to that of normal intact mammary glands. This was accomplished by the perfusion of excised mammary glands with high concentrations of blood glucose.

2. It has been possible to convert the glycogen formed during perfusion

to lactose by means of incubation of the perfused tissues. This does not in any way prove that this is the path of lactose formation in mammary glands, but it appears to be a possible mode of metabolism by which several compounds could be converted to lactose.

3. Glycogen could not be increased in incubating tissue breis from glucose, lactic acid, pyruvic acid and citric acid individually, and in combinations of these, as well as in the presence of added glycogen.

4. Lactose was formed during incubation of mammary tissue slices with glucose, glucose and lactic acid, maltose, and glycogen.

5. Lactic acid was formed in incubating tissue alone, as well as from added glucose, maltose, glycogen, pyruvic acid, and citric acid.

## REFERENCES

(1) Barker, S. B., and Summerson, W. H. The Colorimetric Determination of Lactic Acid in Biological Material. Jour. Biol. Chem., 138: 535–554. 1941.

(2) Barrenschen, H. K., and Alders, N. A. Über den kohlenhydratestoffwechsel der ruhenden und tatigen milchdruse. Biochem. Ztschr., 252: 97–112. 1932.

(3) Bert, M. P. Sur l'origine du sucre de lait. Compt. Rend. Acad. Sci., 98: 775–777. 1884.

(4) Blackwood, J. H., and Stirling, J. D. The Absorption of Milk Precursors off the Mammary Gland. 2. The Relation of Blood Sugar Absorption to Lactose Secretion. Biochem. Jour., 26: 362–368. 1932.

(5) Bottomley, A. C., Folley, S. J., Walker, F. H. A., Scott, M., and Watson, H. M. Effect of Subcutaneous Implantation of Adrenalin Tablets on the Blood Sugar and Milk Composition in Lactating Ruminants. Endocrinology, 1: 287–295. 1939.

(6) Brown, W. R., Petersen, W. E., and Gortner, R. A. The Decrease in the Lactose Content of Milk Following the Production of Artificial Hypoglycemia. Jour. Dairy Sci., 19: 147–154. 1936.

(7) Brown, W. R., Petersen, W. E., and Gortner, R. A. The Effect of Intravenous Injections of Sugar upon the Lactating Bovine. Jour. Dairy Sci., 19: 177–184. 1936.

(8) Brown, W. R., Petersen, W. E., and Gortner, R. A. Intra-mammary Duct Injections in the Study of Lactose Formation. Jour. Dairy Sci., 19: 243–256. 1936.

(9) Bucciardi, O. L' azione dell' pilocarpina sulla secrezione e sulla quantita sio nil latte. Soc. Ital. di Biol. Sper. Bol., 3: 527–530. 1928.

(10) Cori, G. T., and Cori, C. F. The Activating Effect of Glycogen on the Enzymatic Synthesis of Glycogen from Glucose-1-phosphate. Jour. Biol. Chem., 131: 397–398. 1939.

(11) Friedemann, T. E., and Haugen, G. E. Pyruvic Acid. II. The Determination of Keto Acids in Blood and Urine. Jour. Biol. Chem., 147: 415–442. 1943.

(12) Good, C. A., Kramer, H., and Somogyi, M. The Determination of Glycogen. Jour. Biol. Chem., 100: 485–491. 1933.

(13) Gowan, J. W., and Tobey, E. R. Studies on Milk Secretion. The Influence of Inanition. Jour. Gen. Physiol., 15: 45–66. 1931.

(14) Gowen, J. W., and Tobey, E. R. On the Mechanism of Milk Secretion. The Influence of Insulin and Phloridzin. Jour. Gen. Physiol., 15: 67–85. 1931.

(15) Graham, W. R. The Utilization of Lactic Acid by the Lactating Mammary Gland. Jour. Biol. Chem., 122: 1–9. 1937.

(16) GRAHAM, W. R., HOUCHIN, O. B., PETERSEN, W. E., AND TURNER, C. W. The Efficiency of the Mammary Gland in the Production of Milk. Amer. Jour. Physiol., 150: 122–126. 1938.

(17) GRAHAM, W. R., JONES, T. S. G., AND KAY, H. D. The Precursors in Cow's Blood of Milk Fat and other Milk Constituents. Roy. Soc. London Proc., Sec. B., 120: 330–346. 1936.

(18) GRAHAM, W. R., JR., KAY, H. D., AND MCINTOSH, R. A. A Convenient Method for Obtaining Bovine Arterial Blood. Roy. Soc. London Proc., Sec. B., 120: 319–329. 1936.

(19) GRANT, G. A. The Metabolism of Galactose. II. The Synthesis of Lactose by Slices of Active Mammary Gland *in Vitro*. Biochem. Jour., 29: 1905–1909. 1935.

(20) GRANT, G. A. The Metabolism of Galactose. III. 1. Lactose Synthesis from (a) A Glucose-galactose Mixture, (b) Phosphoric Esters, by Slices of the Active Mammary Gland *in Vitro*. 2. The Effect of Prolactin on Lactose Synthesis by the Mammary Gland. Biochem. Jour., 30: 2027–2035. 1936.

(21) GREEN, A. A., CORI, G. T., AND CORI, C. F. Crystalline Phosphorylose. Jour. Biol. Chem., 142: 447–448. 1942.

(22) GUISTI, L., AND RIETTI, C. T. Accion de la Insulina sobre la Composition de la Leche. Rev. de las Assoc. Med. Agr. Soc. d Biol., 36: 279–283.

(23) HESSE, E. Uber die Milchzuckers in der Milchdruse. Biochem. Ztschr., 138: 441–449. 1923.

(24) HODGSON, R. E., RIDDELL, W. H., AND HUGHES, J. S. Factors Influencing the Blood Sugar Level of Dairy Cattle. Jour. Agr. Res., 44: 357–365. 1932.

(25) JONES, T. S. G. Blood Constituents and Milk Secretion as Influenced by Thyroxine. Soc. Chem. Indus. Jour., London, 54: 928. 1935.

(26) LINTZEL, W. Untersuchungen uber den Chemismusder Milchfettbildung in Abhangigkeit von der Futterung. Ztschr. f. Zucht., Sec. B., 29: 219–242. 1934.

(27) MACCHAIRULO, O. La Formazine del lattosio nella Mammella Funzioante Sotto L'azine Dell' Insulin. Soc. Ital. di Biol. Sper. Bol., 3: 973–976.

(28) MARSCHALL, F. H. A., AND KIRKNESS, J. M. On the Formation of Lactose. Biochem. Jour. 2: 1–6. 1907.

(29) MICHLIN, A., AND LEWITOW, L. Uber die Synthesis von Lactose in Milchdrüse. Biochem. Ztschr., 271: 448–454. 1934.

(30) MOORE, B., AND PARKER, W. H. A Study of the Effects of Complete Removal of the Mammary Glands. Amer. Jour. Physiol., 4: 239–242. 1900.

(31) NITZESCU, I. I. Action des Sucres in Injections Intraveineuses sur la Secretion du Lait. Soc. de Biol. Compt. Rend., 93: 1319–1321. 1925.

(32) NITZESCU, I. I., AND NICOLEAN, O. L'action de la Insuline sur la Secretion du Lait. Soc. de Biol. Compt. Rend., 91: 1462–1465. 1924.

(33) OVERMAN, O. R., AND WRIGHT, K. E. The Effect of Inanition upon the Yield and the Composition of Cow's Milk. Jour. Agr. Res., 35: 637–644. 1937.

(34) PATON, D. N., AND CATHCART, E. P. On the Mode of Production of Lactose in the Mammary Gland. Jour. Physiol., 42: 179–188. 1911.

(35) PETERSEN, W. E., AND BOYD, W. L. Glucose Infusion Through the External Pudic Artery. Soc. Expt. Biol. and Med. Proc., 37: 539–542. 1937.

(36) PETERSEN, W. E., HEWITT, E. A., BOYD, W. L., AND BROWN, W. R. Artificially Induced Hypoglycemia and Hypocalcemia in the Cow and the Relationship to Parturient Paresis or Milk Fever. Amer. Vet. Med. Assoc. Jour., 79: 217–227. 1931.

(37) PETERSEN, W. E., AND SHAW, J. C. *In Vitro* Synthesis of Lactose. Sci., 86: 398. 1937.

(38) PETERSEN, W. E., AND SHAW, J. C. Relation of Lactic Acid and Glucose of the Blood and Glycogen in the Mammary Gland to Milk Secretion. JOUR. DAIRY SCI., **21**:168. 1938.

(39) PETERSEN, W. E., SHAW, J. C., AND VISSCHER, M. B. A Technique for Perfusing Excised Bovine Mammary Glands. JOUR. DAIRY SCI., **24**: 139–146. 1941.

(40) POWELL, R. C., AND SHAW, J. C. The Non-utilization of Lactic Acid by Lactating Mammary Gland. Jour. Biol. Chem., **146**: 207–210. 1942.

(41) REINEKE, E. P., WILLIAMSON, M. B., AND TURNER, C. W. Utilization of the Glycoprotein of the Blood Plasma by the Lactating Mammary Gland. Jour. Biol. Chem., **138**: 83–90. 1941.

(42) ROBISON, R., AND ROSENHEIM, A. H. XCV. Calcification of Hypertonic Cartilage *in Vitro*. Biochem. Jour., **28**: 684–698. 1934.

(43) ROHMANN, F. Uber die Bildung des Milchzuckers in der Milchdruse. Biochem. Ztschr., **93**: 237–252. 1919.

(44) SCHULTZ, A. S., AND AKIN, L. Fermentation of Maltose. Amer. Chem. Soc. Jour., **61**: 291–294. 1939.

(45) SCOTT, M., AND WEST, E. S. Determination of Glucose, Galactose, and Lactose in a Mixture. Soc. Expt. Biol, and Med. Proc., **34**: 52–54. 1936.

(46) SHAFFER, P. A., AND SOMOGYI, M. C. Copper-iodometric Reagents for Sugar Determinations. Jour. Biol. Chem., **100**: 695–713. 1933.

(47) SHAW, J. C., BOYD, W. L., AND PETERSEN, W. E. Blood Glucose and Lactic Acid in Milk Secretion. Soc. Expt. Biol. and Med. Proc., **38**: 579–585. 1938.

(48) SOMOGYI, M. Solubility and Preparation of Glycogen. Jour. Biol. Chem., **104**: 245–253. 1934.

(49) SVANBERG, C. Enzymatische versuche mit milchdrusen. Hoppe-Seylers Ztschr. f. Physiol. Chem., **188**: 207–218. 1930.

# NEGATIVE PRESSURE AND NURSING BY CALVES[1]

VEARL R. SMITH AND W. E. PETERSEN
*Dairy Division, University of Minnesota*

The question of the amount of negative pressures a calf develops in nursing arose in a study of fundamentals involved in mechanical milking. The report of Krzywanek and Brüggemann (1) dealing with measurements of the forces exerted in sucking on specially constructed nipples was the only reference to the subject found in the literature. These workers found that calves, in nursing, exerted pressures up to 2.0 inches Hg and negative pressures down to 11.4 inches Hg. They expressed the opinion that the negative pressures developed by calves sucking the specially constructed nipple were greater than would occur in normal nursing.

This report deals with the results obtained in a study attempting to ascertain the maximum negative pressures a calf can develop in nursing, the relative negative pressures developed in nursing naturally, and whether or not a calf can obtain milk without development of negative pressure.

## PROCEDURE

To ascertain the negative pressure a calf can develop during the nursing act, a rubber tube, with an overall diameter of one-quarter inch, was connected to a vacuum gauge and inserted between the teat of the cow and tongue of the calf while sucking.

An apparatus was constructed of a rubber calf nipple, rubber tubing and a vacuum gauge by which the negative pressure could be determined. The opening of the calf nipple was enlarged to eliminate resistance. The nipple was connected to an aluminum tube which was placed in a container for milk. The standard vacuum gauge was attached to the tube for conveyance of milk and the nipple by means of a T-tube.

Twenty calves ranging in age from 2 to 102 days of age were readily induced to obtain milk from this apparatus. After they had sucked milk for a while, the tube leading to the milk was pinched off distally to the point of connection of the vacuum gauge; and the maximum negative pressure developed was observed.

In order to ascertain whether or not a calf can obtain milk without suction (negative pressure), a small aluminum tube was inserted into the calf's mouth above the tongue while nursing. This tube prevented the creation of a negative pressure in the mouth. This experiment was tried on calves nursing cows as well as the apparatus previously described. When sucking on the artificial nipple, whether or not the calf obtained milk under those

Received for publication December 1, 1944.

[1] Scientific Journal Series Paper No. 2208, Minnesota Agricultural Experiment Station.

conditions was easily observed through a glass tube inserted into the rubber tube leading to the milk. In order to determine this fact when calves nursed cows, it was necessary in one case to perform an esophagotomy and cannulate the esophagus. Any milk obtained would flow through the cannula.

## RESULTS

The wrapping of the tongue around the teat when compressed against the upper jaw forms an air-tight compartment of the oral cavity. It was observed that negative pressure may be created either by enlargement of the oral cavity by separation of the jaws or a backward drawing of the tongue,

TABLE 1

*Comparison of maximum negative pressure developed by calves sucking cows and artificial nipples*

| Identification of animal | Age at time of experiment | Maximum negative pressure | | Cow nursed |
|---|---|---|---|---|
| | | Nipple | Cow | |
| | *days* | *inches mercury* | | |
| B556 | 43 | | 10 | 804 |
| B556 | 46 | | 16 | A15 |
| B556 | 45 | 15 | | |
| 669 | 24 | | 12 | 493 |
| 669 | 26 | 14 | | |
| 670 | 23 | | 12 | 493 |
| 670 | 26 | 15 | | |
| 841 | 34 | | 11 | 805 |
| 841 | 51 | 18 | | |
| 663 | 19 | | 9 | 805 |
| 663 | 21 | 16 | | |
| B157 | 36 | | 10 | 493 |
| B157 | 39 | 12 | | |
| B377 | 35 | | 12 | 805 |
| B377 | 51 | 14 | | |
| B388 | 2 | | 13 | 493 |
| B388 | 4 | 14 | | |
| B382 | 6 | | 13 | A12 |
| B382 | 6 | 17 | | |
| 659 | 85 | | 10 | A15 |
| 659 | 81 | 15 | | |

which increases the dimensions of the laryngeal cavity. Both of these actions contribute to the creation of negative pressure in normal sucking.

A comparison of the negative pressures developed by calves sucking cows and artificial nipples is presented in table 1. The maximum negative pressures developed by sucking nipples ranged from 12 to 18 inches Hg negative pressure as compared to a range of 9 to 16 inches Hg negative pressure when the same calves sucked cows. Negative pressure levels obtaind from ten additional calves sucking rubber nipples are presented in table 2. These values vary from 14 to 17 inches Hg negative pressure.

When the development of negative pressure was prevented by the insertion of a tube into the mouth, no milk could be obtained by nursing on the special apparatus as evidenced by observation in the glass tube through

TABLE 2

*Maximum negative pressures developed by calves nursing on a special apparatus*

| Identification of animal | Age at time of experiment | Maximum negative pressure |
|---|---|---|
| | *days* | *inches mercury* |
| E477 | 58 | 16 |
| A54 | 10 | 14 |
| E480 | 30 | 14 |
| E481 | 17 | 14 |
| E482 | 17 | 16 |
| 835 | 21 | 15 |
| B141 | 102 | 14 |
| B144 | 10 | 15 |
| E483 | 11 | 16 |
| B411 | 15 | 17 |

which the milk must flow. These results were confirmed when the calf in which the esophagus had been cannulated nursed a cow. Milk flowed freely from the cannula in the esophagus when the calf nursed normally. When the tube was inserted in the mouth so as to prevent the development of any negative pressure, no milk was obtained.

## DISCUSSION

It will be noted from the results in table 1 that the negative pressures developed on the nipple were invariably greater than the values obtained when the calves sucked cows. This would indicate that the calf can readily develop sufficient negative pressure to overcome normal resistance of the teat sphincter.

The amount of negative pressure required for opening of the teat meatus varies with cows and the individual quarters of cows, and is dependent on the ease or hardness of milking. Reference to table 1 shows that measurements of negative pressure were made on calf B556 while sucking two different cows. On cow 804, an easy milker, only 10 inches of Hg negative pressure was developed as compared to 16 inches on cow A15, a hard milker.

## CONCLUSIONS

From the observations made in these experiments, it is apparent that the calf can develop a negative pressure as great as, and more often in excess of, that required in the milking machine to milk hard-milking cows. It is further demonstrated that the calf cannot obtain milk by nursing when a tube is inserted in the mouth to prevent the development of negative pressure. This tube did not interefere with the action of the tongue or jaws, and therefore it is presumed that the calf cannot obtain milk from the teat by the compressing action of the jaws.

## REFERENCE

(1) KRZYWANEK, W., AND BRÜGGEMANN, H. Modellversuche zur Physiologie des Saugaktes. Berlin. Tierärztl. Wchnschr., 20: 710–713. 1930.

# THE VARYING REQUIREMENT FOR CHOLINE FOR NORMAL METABOLISM OF PROTEINS (CASEIN, LACTALBUMIN, AND SOY PROTEIN) WITH PARTICULAR REFERENCE TO THE HEPATICO-RENAL SYNDROME[1, 2]

G. C. SUPPLEE,[3] LORRAINE S. GALL AND JEAN F. CAUL
WITH THE COOPERATION OF L. C. BABCOCK AND ESTHER G. NOHLE
*Bainbridge, New York*

Excessive deposition of fat in the liver and concurrent hemorrhagic degeneration of the kidneys in young animals are seemingly syndromes resulting from imperfect metabolic processes involving choline and/or the methionine and cystine components of the dietary protein. Lecithin and its choline component were first recognized by Best *et al.* (1, 2) as being markedly effective in maintaining normal liver fat levels. Channon *et al.* (5, 6) later reported that casein was also similarly effective, its lipotropic action being greater than that of most of the proteins tested. Tucker and Eckstein (25), and Channon also (7) subsequently presented further data attributing the lipotropic action of proteins to their methionine content. Griffith and Wade (16) recorded the further significant observation that hemorrhagic degeneration of the kidneys occurred concurrently with a high fat content of the liver in young rats on a choline-deficient diet. Their studies (17) also directed attention to the significance of the cystine: methionine ratio of the proteins; these data indicated that the higher the cystine content of the diet, the greater the requirement for choline or its equivalent. Du Vigneaud and associates (10, 11), showed that homocystine in the absence of methionine and choline failed to support growth, and fatty infiltration of the liver occurred; when choline was supplied, however, rapid growth resulted equivalent to that obtained from the feeding of methionine. These observations led to the establishment of further impressive evidence by du Vigneaud *et al.* (12, 13) elucidating the chemical reactions interrelating choline and methione, wherein it was shown that the methyl group or groups present in these substances function reversibly in a transmethylation cycle in the body.

Since these data appear to establish the essentiality of labile methyl for normal metabolism and also seemingly serve as a basis for interrelating the

Received for publication December 2, 1944.

[1] Presented before American Chemical Society, Division of Biological Chemistry, Cleveland, Ohio, April 3, 1944.

[2] From Borden Research Division at Bainbridge, N. Y., prior to abandonment of Biological and Chemical Research Laboratories at that location.

[3] President and Technical Director, Supplee Research Corporation, Bainbridge, New York.

qualitative character of the dietary protein with liver function, it appears that choline, should, other things equal, assume a quantitative rôle in metabolism, compensating to a degree for sub-optimal methionine intake as may occur from low protein dietaries or diets containing protein of low or relatively unavailable methionine content. The present studies were designed to determine the degree to which this quantitative concept could be demonstrated by comparing the extent of functional impairment of liver and kidneys induced by variable amounts of "total" known labile methyl ingested. The indices of functional impairment involving precise analytical estimations considered useful as differentiating criteria were: Liver fat determinations, the amount of uric acid retained in the kidneys, and the concentration of riboflavin in the kidney tissue fractionally determined as "free" and "bound" riboflavin. Rate of growth and the incidence and degree of liver and kidney pathology were likewise used for purposes of correlation. In view of the spontaneous or partial correction of the kidney damage resulting from choline deficiency with time, possibly due to the synthesis of choline within the body (12, 19), the experimental plan provided for the requisite analyses at 4-day intervals through a 12-day period and again after a 28-day period on the test diet. These observation periods were designed to cover the transient period of acute kidney impairment brought about by labile methyl deficiency or by the character of the protein.

Accurate knowledge of the labile methyl intake requisite for the objectives of the study was obtained by calculations based upon the actual methionine content of the test protein consumed and that furnished by supplemental daily doses of pure choline hydrochloride or pure methionine. For record purposes, the labile methyl intake irrespective of source, was reduced to a common basis of expression designated as "choline equivalent," wherein 3.8 parts of methionine were presumed to be equivalent to 1 part of choline (8, 18).

## EXPERIMENTAL

Groups of weanling rats, containing from 4 to 16 animals per group with an equal number of each sex, 40–45 grams in weight were provided with the test diets containing varying percentages of each of 4 purified proteins of known methionine, cystine and choline content (table 1). The daily regimen consisted of a dry basal mixture composed of hydrogenated vegetable oil (Crisco), 3 parts; salt mixture No. 40 (23), 4 parts; cod liver oil (medicinal grade), 2 parts; test protein, 16, 20 or 24 parts; and sucrose to make up to 100 parts. The water-soluble vitamin factors supplied as a daily supplement of pure entities consisted of: 12.5 γ thiamine, 10 γ riboflavin, 10 γ pyroxidine and 50 γ pantothenic acid as the calcium salt, carried in 2 ml. of a standardized solution mixture. Choline or methionine when called for in the experimental plan were likewise fed as daily supplements in standardized solutions mixed with the vitamin supplement.

The quantity of choline or methionine supplement was that amount designed to supply a uniform total daily intake of "choline equivalent," assuming the methyl of the methionine of the protein and that of the supplement, to be biologically equivalent to the same amount of methyl carried by the choline. Since preliminary data had indicated that a total choline equivalent intake per rat per day of at least 12 mg. was necessary for maintaining normal liver fat levels, it was decided to establish the threshold level at 13.5 mg. Accordingly, the choline or methionine supplement varied, depending upon the methionine content of the particular test protein. The method of calculating the choline and methionine supplements is illustrated in the following examples:

For the 16 per cent soy-protein diet, a 5-gram daily food consumption representing 0.8 gram protein, was assumed. Since the methionine content of this protein was 1.42 per cent, the intake would be 11.36 mg.; assuming

TABLE 1

*Methionine, cystine and choline content of test proteins*

| Protein | Total nitrogen | Methionine* | Cystine* | Choline† |
|---|---|---|---|---|
| | % | % | % | *mg./gm.* |
| Labco V. F. casein | 15.40 | 3.10 | 0.32 | None |
| Labco lactalbumin 7HAA | 14.15 | 2.63 | 3.00 | 0.90 |
| Labco lactalbumin Me–3‡ | 15.05 | 2.02 | 2.45 | None |
| Soy protein S–50 | 15.45 | 1.42 | 1.42 | 0.40 |

* Analyses by R. J. Block; methionine by the McCarthy-Sullivan method, and cystine by a modification of the Folin procedure.

† Determinations by Dr. E. A. Prill, using the reinekate method applied to the methanol extract (Jour. Biol. Chem., **144**: 701, 1942).

‡ Soluble lactalbumin (Weech, A. A., Bull. Johns Hopkins Hospital, **70**: 157, 1942).

3.8 mg. methionine to be the biological equivalent of 1 mg. choline, the "choline equivalent" carried by the ingested protein is 2.99 mg., leaving 10.51 mg. choline to be supplied. The amount of the methionine supplement required to furnish an equivalent of 10.51 mg. choline would be 39.93 mg. ($10.51 \times 3.8$). Similar calculations for the 24 per cent casein diet necessitated a daily choline supplement of only 3.7 mg., or a methionine supplement of 14.06 mg. Since actual food consumption was not maintained at the assumed 5-gram daily intake, calculation of the actual choline equivalent intake was made from the food consumption records and uniform daily supplements furnished.

At the end of the observation periods, the groups of animals receiving each of the test dietaries were sacrificed following an 18-hour fast to deplete liver glycogen reserves. Autopsies were made immediately and liver and kidneys subjected to analyses by the following procedures:

*Analytical Methods:*

The total liver lipids (fresh tissue) were determined by the method of Kaplan and Chaikoff (20).

Uric acid retained in the kidneys was determined by modification of the Folin procedure for blood (14). Immediately following removal, both kidneys were quickly blotted, decapsulated, halved, weighed and placed in a test tube containing 10 ml. of boiling N/100 acetic acid for 5 minutes to inactivate the enzyme which converts xanthine to uric acid (3). The contents of the tube were quantitatively transferred to a mortar, ground with washed sand and filtered with mild suction. The filtrate with washings, total volume 23 ml., was neutralized to litmus paper with N NaOH before precipitation of the protein by tungstic acid. After 30 minutes the protein was filtered off and the filtrate diluted to exactly 25 ml. (The residue on the filter paper was oven-dried and saved for the determination of "bound" riboflavin). To 12.5 ml. of the filtrate there was added 0.5 ml. of 2.5 per cent $ZnCl_2$ and 1 ml. of 20 per cent $Na_2CO_3$. The zinc urate thus formed was occluded with the heavier $ZnCO_3$ precipitate. Following centrifuga-

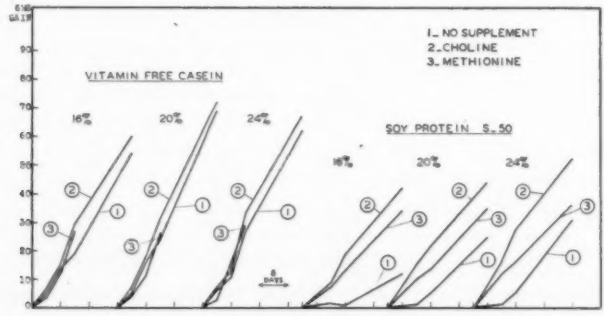



FIG. 1. Growth rate as affected by casein and soy protein at varying percentages of the diet, with and without choline or methionine supplementation.

tion, discarding of the supernate, and drainage, the precipitate was dissolved in 2.0 ml. of urea cyanide; 0.8 ml. uric acid reagent were then added and the blue color permitted to develop for 20 minutes. Before colorimetric comparison with the standard, the blue solution was diluted with water to 5 ml. Inasmuch as kidney uric acid is an excretory product and not a constituent of the kidney tissue, the results are expressed as mg. of uric acid per two kidneys.

The procedure for determining the riboflavin concentration in the kidneys permitted a significant differentiation as "free" and "bound" riboflavin.

For the determination of the "free" fraction, the unused 12.5-ml. portion of the protein-free filtrate remaining from the uric acid determination was used after appropriate dilution, for estimation by the fluorometric method (24).

For the determination of "bound" riboflavin, the oven-dried residue and filter paper retained from the uric acid determination was used. The total

residue and paper were refluxed in the dark with three 50-ml. quantities of 80 per cent acetone for 30 minutes (40 ml. acetone and 10 ml. N/0.25 $H_2SO_4$). The acetone was evaporated from the combined filtrates and washings and the aqueous residue neutralized to litmus with 3.6 N NaOH. Normal $H_2SO_4$ was then added until maximum precipitation occurred, after which the sample was diluted to 100 ml. with water and allowed to stand ½ hour before final adjustment to pH 5.5 using an electric potentiometer. Suspended matter was filtered with mild suction through a sintered glass crucible of 7.5-micron porosity. The riboflavin content of the clear filtrate was determined fluorometrically by the same procedure used for determining "free" riboflavin.

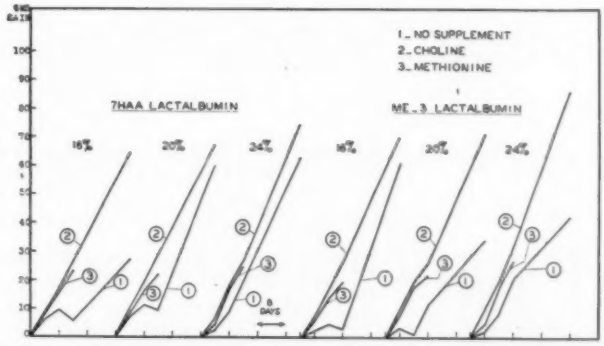



FIG. 2. Growth rate as affected by lactalbumins at varying percentages of the diet, with and without choline or methionine supplementation.

A retarded growth rate or weight loss in the absence of choline or methionine supplements resulted from each of the proteins with the exception of casein, during the first 8- to 12-day period (figures 1 and 2; see table 2 for average daily choline equivalent intake). A mortality rate of 50 per cent to 75 per cent among the animals receiving the unsupplemented albumins and soy protein was not uncommon during the first 12 days; no deaths occurred in the groups receiving casein either with or without supplements, nor in the groups receiving the other proteins when supplemented with choline or methionine.

The lipotropic action of the unsupplemented proteins was increased to only a minor degree and irregularly so by increasing the protein level from 16 to 24 per cent of the diet (table 2). The liver fat levels resulting from the unsupplemented low soy protein and lactalbumin diets generally increased during the 28-day observation period. The unsupplemented casein diets were markedly more effective in maintaining low liver fat concentrations than any of the other proteins. Choline supplementation to a degree which provided a total choline equivalent intake of about 13–15 mg. per day

TABLE 2

*Liver fat as affected by different proteins at varying percentages of the diet with and without choline or methionine supplementation*

| Dietary protein | % | Supplement | After 4 days | | After 8 days | | After 12 days | | After 28 days | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Choline equiv. | Liver fat | Choline equiv. | Liver fat | Choline equiv. | Liver fat | Choline equiv. | Liver fat |
| | | | *mg./day* | % | *mg./day* | % | *mg./day* | % | *mg./day* | % |
| V. F. casein | 16 | None | 6.6 | 9.2 | 10.1 | 12.6 | 8.2 | 15.6 | 9.5 | 12.3 |
| " " " | 20 | " | 7.5 | 7.2 | 9.8 | 10.1 | 9.0 | 11.7 | 12.4 | 6.0 |
| " " " | 24 | " | 8.7 | 5.3 | 11.8 | 6.1 | 11.6 | 5.4 | 13.1 | 6.0 |
| " " " | 16 | Choline | 12.0 | 4.9 | 14.3 | 4.6 | 14.4 | 5.5 | 15.7 | 5.9 |
| " " " | 20 | " | 10.6 | 5.1 | 14.6 | 6.1 | 13.7 | 5.4 | 15.1 | 5.4 |
| " " " | 24 | " | 9.9 | 4.1 | 13.1 | 5.1 | 13.8 | 6.6 | 15.6 | 3.9 |
| " " " | 16 | Methionine | 12.5 | 4.2 | 14.3 | 8.7 | 13.7 | 13.8 | ..... | ..... |
| " " " | 20 | " | 12.1 | 6.1 | 14.5 | 6.6 | 13.3 | 9.2 | ..... | ..... |
| " " " | 24 | " | 9.9 | 5.1 | 15.0 | 5.5 | 13.9 | 5.1 | ..... | ..... |
| Soy S–50 | 16 | None | ..... | ..... | 3.3 | 18.6 | 2.8 | 23.2 | 3.6 | 28.7 |
| " " | 20 | " | ..... | ..... | 4.5 | 12.6 | 3.6 | 20.2 | 4.3 | 21.1 |
| " " | 24 | " | ..... | ..... | 5.0 | 7.1 | 5.3 | 19.9 | 4.4 | 31.3 |
| " " | 16 | Choline | ..... | ..... | 15.0 | 5.6 | 19.1 | 8.6 | 15.1 | 8.4 |
| " " | 20 | " | ..... | ..... | 17.1 | 5.8 | 15.6 | 8.6 | 15.3 | 6.5 |
| " " | 24 | " | ..... | ..... | 16.9 | 7.2 | 15.5 | 6.3 | 15.4 | 8.3 |
| " " | 16 | Methionine | ..... | ..... | 16.7 | 16.5 | 14.1 | 15.9 | 7.8 | 28.5 |
| " " | 20 | " | ..... | ..... | 17.8 | 17.1 | 14.2 | 11.3 | 9.6 | 27.3 |
| " " | 24 | " | ..... | ..... | 17.1 | 10.8 | 14.5 | 15.2 | 10.9 | 27.8 |
| Lactalb. 7HAA | 16 | None | 5.4 | 19.5 | 5.9 | 40.6 | 4.9 | 41.4 | 5.6 | 29.1 |
| " " | 20 | " | 7.0 | 16.5 | 8.0 | 15.1 | 6.2 | 18.7 | 9.3 | 23.6 |
| " " | 24 | " | 9.0 | 13.5 | 10.8 | 14.7 | 9.8 | 15.2 | 14.4 | 18.8 |
| " " | 16 | Choline | 13.5 | 6.4 | 15.7 | 7.4 | 15.1 | 8.4 | 16.2 | 12.3 |
| " " | 20 | " | 13.6 | 10.1 | 15.3 | 7.0 | 15.1 | 8.0 | 16.9 | 8.3 |
| " " | 24 | " | 14.0 | 4.3 | 14.9 | 7.0 | 15.1 | 5.6 | 18.3 | 6.1 |
| " " | 16 | Methionine | 13.6 | 8.4 | 15.4 | 8.2 | 14.8 | 10.5 | ..... | ..... |
| " " | 20 | " | 12.6 | 12.5 | 15.2 | 11.1 | 14.2 | 11.6 | ..... | ..... |
| " " | 24 | " | 12.9 | 6.8 | 14.8 | 9.0 | 14.1 | 9.5 | ..... | ..... |
| Lactalb. ME–3 | 16 | None | 4.6 | 18.8 | 4.4 | 20.5 | 2.0 | 12.9 | 6.5 | 32.1 |
| " " | 20 | " | 5.0 | 17.7 | 5.1 | 13.8 | 4.4 | 19.4 | 6.0 | 26.9 |
| " " | 24 | " | 5.9 | 17.6 | 6.9 | 18.6 | 8.7 | 25.9 | ..... | ..... |
| " " | 16 | Choline | 13.3 | 6.1 | 14.6 | 6.6 | 15.1 | 7.3 | 15.7 | 6.4 |
| " " | 20 | " | 13.4 | 6.5 | 15.3 | 5.2 | 15.3 | 5.7 | 15.9 | 8.0 |
| " " | 24 | " | 13.8 | 7.4 | 14.4 | 6.3 | 15.9 | 6.4 | 17.7 | 6.8 |
| " " | 16 | Methionine | 13.3 | 7.0 | 13.9 | 7.9 | 15.0 | 8.7 | ..... | ..... |
| " " | 20 | " | 13.7 | 7.9 | 14.2 | 11.3 | 14.7 | 13.8 | ..... | ..... |
| " " | 24 | " | 12.1 | 6.8 | 14.3 | 10.9 | 16.1 | 8.4 | ..... | ..... |

nearly always maintained the liver fat content within the normal range of about 5–7 per cent, in contrast to levels as high as 40 per cent in those animals receiving the same protein unsupplemented. The methionine supplement provided at levels furnishing 13 to 15 mg. total choline equivalent intake per day, while exhibiting measurable lipotropic action, was substantially less effective than choline furnished at the same calculated labile methyl intake. Marked variations were found in the liver fat concentration of animals receiving substantially the same labile methyl intake as carried by the unsupplemented protein; such variations were greatly reduced when the choline or methionine supplements were provided. The scatter-graph (figure 3) shows the correlation of the liver fat values with the choline equivalent intake for the 4-, 8- and 12-day periods. The inverse relation-

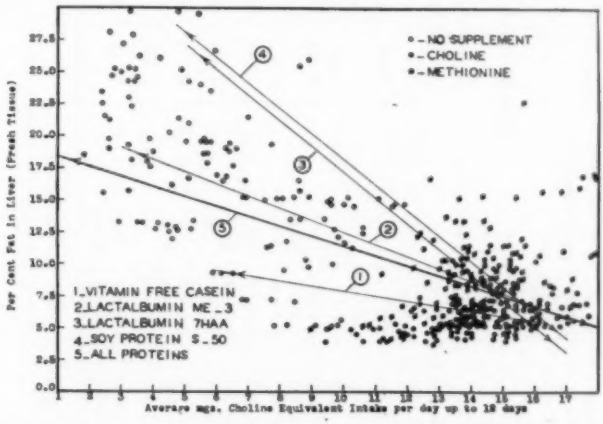



FIG. 3. Scatter graph with statistically determined correlation between choline equivalent intake per day and liver fat level, for young rats receiving different proteins at varying percentages of the diet, with and without choline or methionine supplementation.

ship between the amount of the labile methyl (choline equivalent) intake and the liver fat level which occurred with each of the proteins and for all proteins combined is well shown by the slopes of the straight lines determined by the method of least squares.

Data correlating the choline equivalent intake with the incidence of gross kidney pathology and retention of uric acid in the kidneys are shown in table 3; results from the different protein levels at the 4-, 8- and 12-day observation periods have been pooled to facilitate comparison. The incidence of kidney damage from the unsupplemented casein was only slightly over 10 per cent, whereas from 78 to 87 per cent of the kidneys from the animals receiving the other unsupplemented test proteins were grossly pathological. Macroscopic evidence of kidney damage was not found in any of the groups receiving the choline supplement, and in only a few cases from

TABLE 3

*Incidence of kidney pathology and variation in uric acid content of kidneys as affected by different dietary proteins, with and without choline or methionine supplementation*

| Dietary protein | Supplement | Normal kidneys* | | | | | Pathological kidneys* | | | | | Kidneys of survivors after 28 days | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | % | Ave. wt. | Uric acid in kidneys | | | % | Ave. wt. | Uric acid in kidneys | | | Ave. wt. | Uric acid in kidneys |
| | | | | Ave. | S.D. | P.E. | | | Ave. | S.D. | P.E. | | |
| | | | *gm.* | *mg.* | *mg.* | | | *gm.* | *mg.* | *mg.* | | *gm.* | *mg.* |
| V. F. casein | None | 89.6 | 0.71 | 0.018 | 0.006 | ± 0.0007 | 10.4 | 0.75 | 0.023 | 0.005 | ± 0.0018 | 1.41 | 0.018 |
| " " " | Choline | 100.0 | 0.65 | 0.014 | 0.003 | ± 0.0003 | None | ..... | ..... | ..... | ..... | ..... | 0.016 |
| " " " | Methionine | 100.0 | 0.72 | 0.017 | 0.004 | ± 0.0005 | None | ..... | ..... | ..... | ..... | ..... | ..... |
| Soy S–50 | None | 14.3 | 0.64 | 0.020 | 0.005 | ± 0.0007 | 85.7 | 1.03 | 0.029 | 0.007 | ± 0.0012 | 0.99 | 0.022 |
| " " | Choline | 100.0 | 0.70 | 0.013 | 0.003 | ± 0.0003 | None | ..... | ..... | ..... | ..... | 1.02 | 0.017 |
| " " | Methionine | 93.7 | 0.71 | 0.018 | 0.004 | ± 0.0005 | 6.3 | 1.13 | 0.032 | 0.003 | ± 0.0013 | 0.98 | 0.018 |
| Lactalb. 7HAA | None | 21.7 | 0.68 | 0.018 | 0.004 | ± 0.0007 | 78.3 | 1.12 | 0.032 | 0.007 | ± 0.0011 | 1.30 | 0.018 |
| " " | Choline | 100.0 | 0.75 | 0.016 | 0.003 | ± 0.0003 | None | ..... | ..... | ..... | ..... | 1.47 | 0.014 |
| " " | Methionine | 94.4 | 0.70 | 0.019 | 0.005 | ± 0.0006 | 5.6 | 1.04 | 0.026 | ..... | ..... | ..... | ..... |
| Lactalb. ME–3 | None | 13.1 | 0.60 | 0.022 | 0.003 | ± 0.0006 | 86.9 | 1.05 | 0.029 | 0.006 | ± 0.0009 | 1.24 | 0.019 |
| " " | Choline | 100.0 | 0.71 | 0.014 | 0.004 | ± 0.0005 | None | ..... | ..... | ..... | ..... | 1.37 | 0.017 |
| " " | Methionine | 97.0 | 0.70 | 0.017 | 0.004 | ± 0.0005 | 3.0 | 1.26 | 0.036 | ..... | ..... | ..... | ..... |
| Stock ration | None | 100.0 | 0.61 | 0.016 | 0.005 | ± 0.0009 | None | ..... | ..... | ..... | ..... | ..... | ..... |

* Examination periods at 4, 8 and 12 days.

those groups receiving the methionine supplement. A higher amount of uric acid was found in the pathological kidneys; likewise, higher amounts were generally found in the kidneys of those animals not receiving the choline supplement even though pathological conditions were not observed. That the higher uric acid retention in the enlarged pathological kidneys is not due to greater size per se, seems apparent from the fact that low values were found in normal or healed organs of equal or greater size at the 28-day observation period. Methionine was not as effective as choline in maintaining low uric acid concentrations in the kidney tissue. The scatter graphs (figures 4 and 5, 6 and 7) show the efficacy of an increasing choline or choline equivalent intake as a factor affecting the clearance of uric acid from the kidney

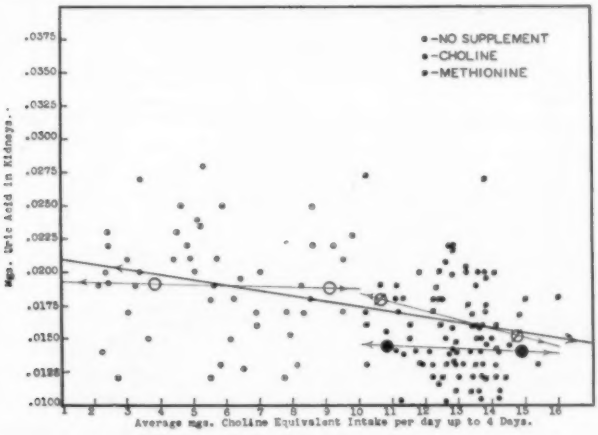



FIG. 4. Scatter graph with statistically determined correlations between choline equivalent intake per day for *4 days* and uric acid in the kidneys of young rats receiving different proteins at varying percentages of the diet, with and without choline or methionine supplementation.

structure. The relationship is the same at each of the observation periods, but the magnitude of the correlation is greatest at the 8- and 12-day periods. This is in conformity with the observation that kidney damage in young animals resulting from choline or labile methyl deficiency reaches the acute stage at about the 10th day and subsides thereafter.

Since all test diets were purine-free, the higher uric acid levels in the kidneys of the animals receiving inadequate choline, may be indicative of greater rate of break-down of liver cells, impairment of liver functions including subnormal destruction of uric acid in the liver, localized impairment of the secretory functions of the kidneys or an interrelationship of impaired functions. The principle site of destruction of uric acid is presumed to be in the liver; little or no destruction takes place in the kidney

and retention in the normal kidney is only transitory incident to absorption and excretion (15). Accordingly, an increase in retention of uric acid in the kidney tissue above the normal would indicate primarily localized functional impairment. Failure in normal elimination would tend to suppress secretory functions and it is known that exposure of the kidney to excessive uric acid concentrations causes damage, affecting the glomerular filtration. Although it is known that glycine and possibly other amino acids stimulate kidney function (4, 9, 21, 26), suppression of the secretory function due to amino acid inadequacy in these experiments is only a matter of conjecture. Glycine insufficiency resulting from impairment of the glycine synthesizing capacity of the liver, (22) or an amino acid inadequacy in the dietaries can be considered only as speculative possibilities at this time.

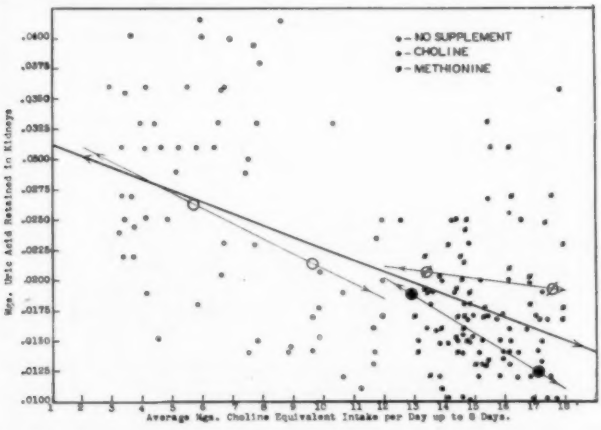

o - NO SUPPLEMENT
o - CHOLINE
ø - METHIONINE
Mgs. Uric Acid Retained in Kidneys
Average Mgs. Choline Equivalent Intake per Day up to 8 Days.

FIG. 5. Scatter graph with statistically determined correlations between choline equivalent intake per day for *8 days* and uric acid in the kidneys of young rats receiving different proteins at varying percentages of the diet, with and without choline or methonine supplementation.

In order to obtain direct evidence indicating impairment of the secretory functions of the kidney, uric acid was injected into appropriately prepared animals receiving the four protein diets at the 16 per cent level, using the same regimen previously described with and without the pre-calculated choline supplement. After 12 days on the test diet, 2.5 mg. uric acid as lithium urate in 0.25 ml. were injected into the heart. The rate of injection was regulated to require about 1 minute; 5 minutes after the needle had been withdrawn, the animals were sacrificed by a blow on the head and the kidneys immediately removed for analyses.

The normal organs of animals receiving the choline supplement showed a greater accumulation of uric acid than those receiving the unsupplemented protein (table 4). This may seem paradoxical in comparison with the results

TABLE 4

*Retention of uric acid in kidneys following injection of 2.5 mg. in the heart, as affected by dietary protein, with and without choline supplementation*

| Dietary protein | Supplement | Kidney wt. | Uric acid in kidneys | | |
|---|---|---|---|---|---|
| | | | Ave. | S.D. | P.E. |
| | | *gm.* | *mg.* | *mg.* | |
| V. F. casein | None | 0.82 | 0.101 | 0.043 | ± 0.008 |
| V. F. casein | Choline | 0.74 | 0.159 | 0.058 | ± 0.009 |
| Soy S–50 | None | 1.05 | 0.076 | 0.022 | ± 0.004 |
| Soy S–50 | Choline | 0.65 | 0.115 | 0.062 | ± 0.010 |
| Lactalb. 7HAA | None | 1.04 | 0.063 | 0.021 | ± 0.004 |
| Lactalb. 7HAA | Choline | 0.68 | 0.129 | 0.064 | ± 0.011 |
| Lactalb. ME–3 | None | 1.09 | 0.050 | 0.024 | ± 0.003 |
| Lactalb. ME–3 | Choline | 0.70 | 0.155 | 0.093 | ± 0.016 |

obtained wherein the retention of endogenous uric acid was relatively less in the normal kidneys from animals receiving the choline supplement. The reversed relationship may be explained as follows: With an unimpaired filtration function in the normal kidneys of the choline supplemented animals, the uric acid level of the glomerular filtrate would tend to promptly come to equilibrium with the high uric acid level of the blood following injection. Also, the elevated uric acid level in the glomerular filtrate passing through the tubules may have been sufficiently toxic to damage temporarily tubular secretion or resorption from this source, thus causing delayed elimination (21). The lower uric acid concentrations following injection, in the kidneys

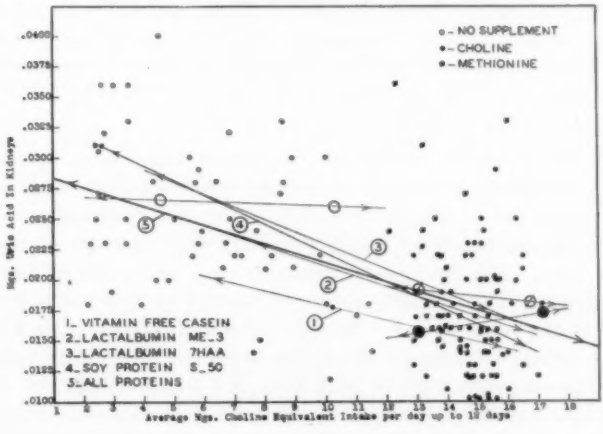



FIG. 6. Scatter graph with statistically determined correlations between choline equivalent intake per day for *12 days* and uric acid in the kidneys of young rats receiving different proteins at varying percentages of the diet, with and without choline or methionine supplementation.

of the animals not receiving the choline supplement, may likewise rationally be explained by impairment of the glomerular filtration previously induced by the choline deficient diets. The relatively higher uric acid values found in the kidneys of the uninjected animals receiving no labile methyl supplement might be predicated upon the assumption of an insidious impairment of the secretory function resulting in a higher retention of endogenous uric acid.

Further evidence of impairment of kidney function was revealed by the differences in the riboflavin concentration in the kidneys of animals receiving the supplemented and unsupplemented diets. The various requisite groups of animals were prepared as usual with each of the test proteins at the 16 per cent level. After 12 days, one-half of the groups with and without

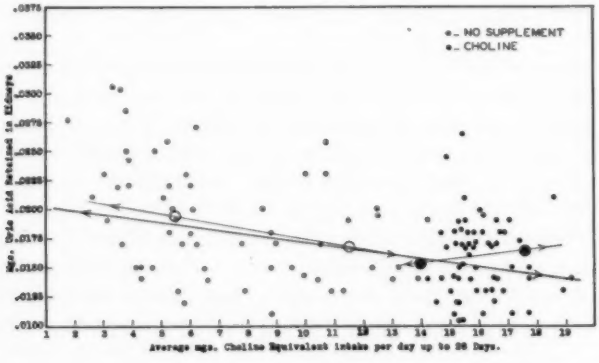



FIG. 7. Scatter graph with statistically determined correlations between choline equivalent intake per day for *28 days* and uric acid in the kidneys of young rats receiving different proteins at varying percentages of the diet, with and without choline supplementation.

the usual pre-calculated choline or methionine supplement were injected with 200 γ riboflavin in 1 ml. of physiological salt solution, using the heart puncture technique. Uniform time intervals were maintained for each animal, the injection requiring about 1 minute, with sacrifice of the animal 5 minutes later. Riboflavin determinations were made immediately on the excised kidneys. The similarly prepared parallel groups not injected were sacrificed at the same time for riboflavin determinations. The kidneys of additional groups of animals were also analyzed after a 28-day feeding period. The concentrations of both free and bound riboflavin were substantially and consistently less in the kidneys of the animals receiving the unsupplemented proteins with inadequate choline or labile methyl; a lesser magnitude of difference resulting from the casein diet (table 5; figures 8 and 9). The same relationship prevailed for both the injected and uninjected animals, and is the reverse of the relationship found for uric acid

TABLE 5

*Riboflavin content of rat kidneys as an index of functional efficiency as affected by dietary protein, with and without choline or methionine supplementation*

| Dietary protein | Supplement | Wt. of kidneys 12 days | Riboflavin injected | Riboflavin content of kidneys (γ per gram fresh tissue) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Ave. "free" | After 12 days | | | | After 28 days | | |
| | | | | | P.E. | Ave. "bound" | P.E. | Total | "Free" | "Bound" | Total |
| | | *gm.* | γ | γ | | γ | | γ | γ | γ | γ |
| V. F. casein | None | 0.85 | None | 2.9 | ±0.111 | 16.4 | ±0.89 | 19.3 | 3.6 | 16.1 | 19.7 |
| " " " | Choline | 0.73 | None | 3.8 | ±0.104 | 19.6 | ±1.72 | 23.4 | 3.4 | 18.7 | 22.1 |
| " " " | None | 0.80 | 200 | 33.4 | ±3.290 | 16.6 | ±1.25 | 50.0 | ..... | ..... | ..... |
| " " " | Choline | 0.74 | 200 | 58.1 | ±1.110 | 15.6 | ±1.68 | 73.7 | ..... | ..... | ..... |
| Soy S–50 | None | 1.08 | None | 1.7 | ±0.005 | 9.1 | ±0.49 | 10.8 | 2.9 | 10.9 | 13.8 |
| " " | Choline | 0.64 | None | 4.0 | ±0.113 | 17.5 | ±1.50 | 21.5 | 3.5 | 16.1 | 19.6 |
| " " | Methionine | 0.78 | None | 2.1 | ..... | 13.3 | ..... | 15.4 | 3.1 | 13.2 | 16.3 |
| " " | None | 1.01 | 200 | 10.9 | ±1.200 | 11.4 | ±0.27 | 22.3 | ..... | ..... | ..... |
| " " | Choline | 0.65 | 200 | 48.4 | ±4.270 | 20.9 | ±1.35 | 69.3 | ..... | ..... | ..... |
| Lactalb. 7HAA | None | 1.09 | None | 1.7 | ±0.105 | 9.5 | ±0.69 | 11.2 | 3.0 | 14.1 | 17.1 |
| " " | Choline | 0.67 | None | 3.7 | ±0.101 | 18.5 | ±0.83 | 22.2 | 3.8 | 14.8 | 18.6 |
| " " | None | 0.99 | 200 | 11.0 | ±1.117 | 10.1 | ±0.86 | 21.1 | ..... | ..... | ..... |
| " " | Choline | 0.69 | 200 | 45.2 | ±3.490 | 25.1 | ±1.20 | 70.3 | ..... | ..... | ..... |
| Lactalb. ME–3 | None | 1.16 | None | 1.8 | ±0.081 | ..... | ..... | ..... | 4.2 | 11.0 | 15.2 |
| " " | Choline | 0.70 | None | 3.6 | ±0.096 | 20.7 | ±1.26 | 24.3 | 4.1 | 16.6 | 20.7 |
| " " | None | 1.03 | 200 | 8.1 | ±0.870 | ..... | ..... | ..... | ..... | ..... | ..... |
| " " | Choline | 0.70 | 200 | 33.2 | ±3.110 | 33.6 | ±4.48 | 66.8 | ..... | ..... | ..... |
| Path. kidneys | ..... | 1.13 | None | 1.6 | ±0.049 | 9.6 | ±0.59 | 11.2 | ..... | ..... | ..... |
| Normal kidneys | ..... | 0.76 | None | 3.5 | ±0.142 | 17.9 | ±0.59 | 21.4 | ..... | ..... | ..... |
| Path. kidneys | ..... | 1.16 | 200 | 9.0 | ±0.697 | 10.8 | ±0.55 | 19.8 | ..... | ..... | ..... |
| Normal kidneys | ..... | 0.73 | 200 | 43.2 | ±3.500 | 20.3 | ±1.07 | 63.5 | ..... | ..... | ..... |

retention and liver fat levels. The differences in the riboflavin concentrations at the 28-day period were relatively less than at the 12-day period, which is indicative of spontaneous improvement in the animals not receiving choline supplements. It appears that under the conditions prevailing in these studies, the difference in riboflavin concentration level of the kidneys is a sensitive index of the efficiency of kidney function as affected by the character of the protein intake and the availability and adequacy of labile methyl.

In order to correlate further the efficiency of kidney function as affected by choline deficiency, the rate of clearance and disposal of injected ribo-

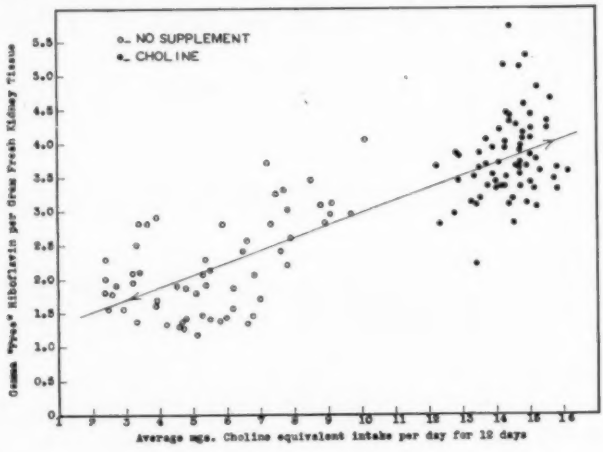



FIG. 8. Scatter graph with statistically determined correlation between choline equivalent intake per day and "free" riboflavin concentration in kidneys of rats receiving different proteins as 16 per cent of the diet, with and without choline supplementation.

flavin was investigated. Groups of animals were properly prepared under the regimen heretofore described with and without the pre-calculated choline supplement, using the soy protein at the 16 per cent level for 10 days. At the end of the preparatory period, 200 $\gamma$ riboflavin in physiological salt solution were injected into the heart. The animals were sacrificed at intervals of 5 minutes, 30 minutes, 2 hours and 4 hours, following injection. Riboflavin determinations were made on the livers, kidneys, blood, that which was recovered from the upper one-third of the intestinal tract, and that excreted or retained in the bladder.

The clearance of the injected riboflavin from the blood stream was more rapid in the animals receiving the choline supplement, likewise, the amount and percentage excreted within a given time was greater from such animals (table 6). Substantially 42 per cent of the amount injected was recovered

in the voided urine or from the bladder of the animals receiving supplemental choline after 2 hours, with no further recovery after 4 hours; whereas, recovery from those animals not receiving choline was 17 per cent after 2 hours and only 26.2 per cent after 4 hours. The "free" riboflavin concentration in the kidneys of the choline-supplemented animals had increased markedly 5 minutes after injection, subsiding rapidly thereafter commensurate with the rate of excretion. On the other hand, the rise in concentration for the animals not receiving choline was of lesser magnitude after 5 minutes and subsided to substantially the same level as that of the uninjected controls after 2 hours. The relationships shown are in con-

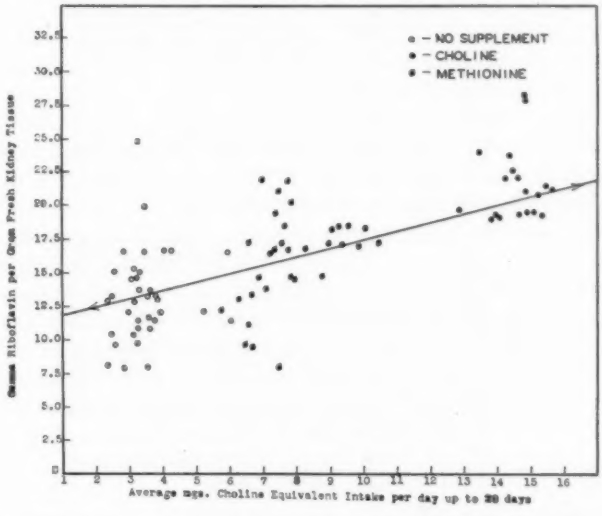

o – NO SUPPLEMENT
• – CHOLINE
* – METHIONINE
Gamma Riboflavin per Gram Fresh Kidney Tissue
Average mgs. Choline Equivalent Intake per day up to 20 days

FIG. 9. Scatter graph with statistically determined correlation between choline equivalent intake per day and riboflavin concentration in the kidneys for rats receiving soy protein S–50 at varying percentages of the diet, with and without choline or methionine supplementation.

formity with the comparative rates of excretion from the two groups and seem to indicate clearly previous impairment of excretory function resulting from inadequate choline or labile methyl.

The unexcreted riboflavin tends to accumulate in the liver. The rise in concentration 5 minutes after injection was substantially the same for both the supplemented and unsupplemented groups when computed to the whole liver basis, fat-free. However, this accentuated concentration was reduced more rapidly in the choline supplemented animals, conforming to the faster rate of excretion, than it was in the animals not receiving choline.

Secretion of riboflavin in the upper portion of the intestinal tract was observed in practically all cases 5 minutes after injection; recoveries varied

TABLE 6

*Rate of excretion and disposition of injected riboflavin as an index of variation in kidney and liver functions induced by choline supplementation of the dietary protein*
(Soy protein S–50 at 16%; 200 γ riboflavin injected in the heart)

| Supplement | Time after injection | Excreted and in bladder | | Blood | | Kidneys | | | | Liver | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | γ/ml. | Total | Wt. | "Free" | "Bound" | Total | Wt. | % Total solids | % Fat-free solids | γ/gm. fat-free solids |
| | *min.* | γ | % | | γ | *gm.* | γ | γ | γ | *gm.* | | | |
| None | Control | 0 | ..... | 0 | ..... | 0.91 | 1.4 | 11.3 | 12.7 | 2.98 | 42.6 | 17.5 | 111 |
| Choline | Control | 0 | ..... | 0 | ..... | 0.77 | 3.9 | 17.5 | 21.4 | 2.47 | 30.8 | 25.2 | 110 |
| None | 5 | 10.1 | 5.0 | 3.6 | 11.8 | 1.05 | 10.1 | 13.0 | 23.1 | 2.81 | 40.3 | 16.5 | 184 |
| Choline | 5 | 45.0 | 22.5 | 2.2 | 11.3 | 0.73 | 36.0 | 20.5 | 56.5 | 2.35 | 29.1 | 23.7 | 156 |
| None | 30 | 44.0 | 22.0 | 1.1 | 4.3 | 1.28 | 3.7 | 17.2 | 20.9 | 2.53 | 45.9 | 19.8 | 155 |
| Choline | 30 | 53.4 | 26.7 | 0 | 0 | 0.75 | 17.5 | 15.0 | 32.5 | 2.66 | 29.2 | 23.1 | 121 |
| None | 120 | 34.0 | 17.0 | 0 | 0 | 0.92 | 1.7 | 11.5 | 13.2 | 3.04 | 39.3 | 15.3 | 160 |
| Choline | 120 | 85.4 | 42.7 | 0 | 0 | 0.71 | 10.2 | 12.0 | 22.2 | 2.41 | 30.3 | 23.5 | 143 |
| None | 240 | 52.4 | 26.2 | 0 | 0 | 1.04 | 2.0 | 8.5 | 10.5 | 2.57 | 39.5 | 19.1 | 176 |
| Choline | 240 | 85.2 | 42.6 | 0 | 0 | 0.82 | 9.8 | 11.5 | 21.3 | 2.43 | 29.8 | 22.7 | 144 |

from 6 to 12 γ, with no consistent difference in the amount recovered from the choline-supplemented animals and the controls.

The data presented indicate varying degrees of functional impairment of liver and kidneys induced by differences in the quality and intake of the dietary protein; such differences are, in part at least, seemingly predicated upon the relative adequacy of labile methyl, either that inherent in the protein or that supplied as choline. The cystine: methionine *ratio* of the dietary does not appear to be the primary factor causing the renal pathology heretofore ascribed to the toxicity of cystine. Seemingly, the quantitative intake of methionine or choline is more important than the maintenance of a given cystine: methionine ratio. Such ratios involved in this investigation varied from 1: 0.82 to 1: 9.69. The adverse effects from a low methoinine intake were always prevented by choline. When the "choline equivalent" intake as supplied by methionine or choline was about 12 mg. per day or more, liver and kidney damage was invariably prevented irrespective of the cystine: methionine ratio. Calculated "choline equivalent" intake carried by the proteins[4] and supplements used by Griffith and Wade (17) shows that when the daily "choline equivalent" intake was below about 8 mg., abnormal livers and kidney lesions always occurred to a greater or lesser degree; when the "choline equivalent" intake was about 12 mg. or more, livers were normal and kidney lesions were absent.

The correlation between the quantitative choline intake and the degree of functional impairment indicated by the analytical data and the superiority of choline as such in comparison with the same levels of calculated choline equivalent intake provided as methionine supplements, lends support to the belief that precise quantitative requirement for readily available labile methyl is constantly imposed during metabolism.

## SUMMARY

1. The development of the hepatico-renal syndrome in young rats reported by others as the result of a choline or labile methyl deficiency, is confirmed. The pathological manifestations determinable by analytical methods are: Excessive deposition of fat in the liver seemingly commensurate with the degree of deficiency, increase in uric acid retention in the kidneys, subnormal riboflavin concentrations in the kidney tissue, and decreased rate of excretion.

2. The subnormal riboflavin concentration in the kidneys is seemingly a sensitive indication of impairment of kidney function.

3. Impairment of kidney function as induced by choline or choline equivalent deficiency progresses in severity in young animals during the first 8 to 12 days; a mortality rate of 50 to 100 per cent during this period is not

[4] Albumin, 5.23% methionine and 1.78% cystine; fibrin, 2.59% methionine and 1.51% cystine: E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids and Peptides," A. C. S. Monograph, p. 348 (1943), Reinhold Publishing Co., New York.

uncommon. The condition spontaneously improves in the survivors after about the 12th to 14th day, with gradual resumption of growth. Concurrent reduction in liver fat levels do not take place, however, and may even continue to increase with time unless choline supplements are provided.

4. A daily "choline equivalent" intake of about 12 to 15 mg. is required for the prevention of the hepatico-renal or choline deficiency syndrome in young rats.

5. The amount of choline to be furnished as a supplement for the prevention of the hepatico-renal syndrome varies with the methionine intake carried by the qualitative character and quantitative level of the dietary protein.

6. Under the conditions prevailing in these experiments, dl-methionine furnished as a supplement at levels calculated to yield the same "choline equivalent" intake as was furnished by choline, proved to be less effective than choline itself.

7. Choline as a supplement of low protein dietaries or diets containing protein of low methionine content furnishes readily available labile methyl, seeming essential for normal protein utilization, effecting, other things equal, a methionine sparing action in promoting growth and maintaining normal liver and kidney functions.

8. Casein was found to be superior to the other proteins tested in preventing the hepatico-renal syndrome; in maintaining normal liver function, and in preventing excessive deposition of fat in the liver.

## REFERENCES

(1) Best, C. H., Hershey, J. M., and Huntsman, M. E. Jour. Physiol., **75**: 56. 1932.
(2) Best, C. H., Hershey, J. M., and Huntsman, M. E. Amer. Jour. Physiol., **101**: 7. 1932.
(3) Blauch, M. B., and Koch, F. C. Jour. Biol. Chem., **130**: 455. 1939.
(4) Brühl, H. Arch. f. die Gesam. Physiol., **220**: 380. 1928.
(5) Channon, H. J., and Wilkinson, H. Biochem. Jour., **29**: 350. 1935.
(6) Channon, H. J., Loach, J. V., Loizides, P. A., Manifold, M. C., and Soliman, G. Biochem. Jour., **32**: 976. 1938.
(7) Channon, H. J., Manifold, M. C., and Platt, A. P. Biochem. Jour., **32**: 969. 1938.
(8) Channon, H. J., Manifold, M. C., and Platt, A. P. Biochem. Jour., **34**: 866. 1940.
(9) Christman, A. A., and Mosier, E. C. Jour. Biol. Chem., **83**: 11. 1929.
(10) du Vigneaud, V., Dyer, H. M., and Kies, M. W. Jour. Biol. Chem., **130**: 325. 1939.
(11) du Vigneaud, V., Chandler, J. P., Moyer, A. W., and Keppel, D. M. Jour. Biol. Chem. Proc., **128**: CVIII. 1939. Jour. Biol. Chem., **131**: 57. 1939.
(12) du Vigneaud, V., Chandler, J. P., Cohn, M., Schenck, J. R., and Simmonds, S. Jour. Biol. Chem., **140**: 625. 1941.
(13) du Vigneaud, V. Biol. Symposium, **5**: 234. 1941.
(14) Folin, O. Jour. Biol. Chem., **101**: 111. 1933.
(15) Folin, O., Berglund, H., and Derick, C. Jour. Biol. Chem., **60**: 361. 1924.
(16) Griffith, W. H., and Wade, N. J. Jour. Biol. Chem., **131**: 567. 1939.

(17) GRIFFITH, W. H., AND WADE, N. J. Jour. Biol. Chem., 132: 627. 1940.
(18) GRIFFITH, W. H., AND MULFORD, D. J. Amer. Chem. Soc. Jour. 63: 929. 1941.
(19) JACOBI, H. P., BAUMAN, C. A., AND MEEK, W. J. Jour. Biol. Chem., 138: 571. 1941.
(20) KAPLAN, A., AND CHAIKOFF, I. L. Jour. Biol. Chem., 108: 201. 1935.
(21) LEWIS, H. B., DUNN, M. S., AND DOISY, E. A. Jour. Biol. Chem., 36: 9. 1918.
(22) QUICK, A. J. Jour. Biol. Chem., 92: 65. 1931.
(23) STEENBOCK, H., AND NELSON, E. M. Jour. Biol. Chem., 56: 355. 1923.
(24) SUPPLEE, G. C., BENDER, R. C., AND JENSEN, O. G. Indus. and Engin. Chem. Analyt. Ed., 11: 495. 1939.
(25) TUCKER, H. F., AND ECKSTEIN, H. C. Jour. Biol. Chem., 121: 479. 1937.
(26) WATZADSE, G. Arch. f. die Gesam. Physiol., 219: 694. 1928.

# A COMPARISON OF CHOLINE, BETAINE AND DIMETHYL-AMINOETHANOL IN THE PREVENTION OF THE HEPATICO-RENAL SYNDROME[1]

G. C. SUPPLEE,[2] JEAN F. CAUL, LORRAINE S. GALL AND E. A. PRILL
WITH THE COOPERATION OF L. C. BABCOCK AND ESTHER G. NOHLE
*Bainbridge, New York*

Choline or an equivalent carrier of labile methyl is rapidly becoming recognized as a dietary essential, especially if the available methionine intake is inadequate. Betaine also has been reported to fulfill the requirements for labile methyl to a certain extent, but it is seemingly less effective than an equal weight of choline (1, 4, 7, 9, 12, 14, 18). In a recent paper from these laboratories (16) a series of studies was reported correlating the choline and methionine intake with liver fat levels and impairment of kidney function as determined by uric acid clearance and the relative concentration of riboflavin in kidney tissue. The present report concerns similar studies involving a comparison of choline and betaines with particular reference to the betaine of glycine; preliminary observations from the use of dimethylaminoethanol are also included.

## EXPERIMENTAL

The experimental plan involved the techniques, dietary regimen, analytical methods and controls previously reported (16), including the same four proteins, namely, a purified vitamin-free casein, two purified lactalbumins, and a purified soy protein, each at 16, 20 and 24 per cent levels. Critical observations and analyses were made after 8- and 28-day feeding periods and after longer intervals for those groups used in the comparisons with mature animals. The choline supplements were fed at pre-calculated levels to furnish a total "choline equivalent" intake of 13 to 15 mg. per rat per day, in accordance with the procedure described in the previous paper. The total "choline equivalent" intake therefore includes the calculated choline equivalent carried by the methionine content of the protein as well as that furnished by the choline supplement. The betaine supplement was usually fed at the same level as the choline supplement in order to permit a direct comparison on a weight for weight basis and in the tabulated "choline equivalent" values (tables 1, 2 and 5) betaine was arbitrarily recorded as being equivalent to its weight of choline.

The growth-promoting properties of equal amounts of choline and betaine furnished as supplements with each of the different proteins were sub-

Received for publication December 2, 1944.

[1] From Borden Research Division at Bainbridge, N. Y., prior to abandonment of Biological and Chemical Research Laboratories at that location.

[2] President and Technical Director, Supplee Research Corporation, Bainbridge, N. Y.

stantially the same during the 28-day observation period (figure 1, see table 1 for actual average daily choline equivalent intake). No deaths occurred

TABLE 1

*The comparative lipotropic action of choline, betaine and dimethylaminoethanol*

| Dietary protein | % | Supplement | After 8 days | | After 28 days | |
|---|---|---|---|---|---|---|
| | | | Choline equivalent | Liver fat | Choline equivalent | Liver fat |
| | | | *mg./day* | % | *mg./day* | % |
| V. F. casein | 16 | None | 10.1 | 12.6 | 9.5 | 12.3 |
| " | 20 | " | ..... | ..... | 12.4 | 6.0 |
| " | 24 | " | ..... | ..... | 13.1 | 6.0 |
| " | 16 | Choline | 14.3 | 6.1 | 15.7 | 5.9 |
| " | 20 | " | ..... | ..... | 15.0 | 5.4 |
| " | 24 | " | ..... | ..... | 15.6 | 4.9 |
| " | 16 | Betaine | 14.5 | 6.1 | 15.5 | 6.5 |
| " | 20 | " | ..... | ..... | 14.8 | 6.0 |
| " | 24 | " | ..... | ..... | 16.3 | 4.9 |
| Soy S–50 | 16 | None | 3.2 | 22.2 | 3.6 | 28.7 |
| " | 20 | " | ..... | ..... | 4.3 | 31.1 |
| " | 24 | " | ..... | ..... | 4.4 | 31.3 |
| " | 16 | Choline | 14.2 | 5.6 | 15.1 | 8.5 |
| " | 20 | " | ..... | ..... | 15.3 | 6.5 |
| " | 24 | " | ..... | ..... | 15.4 | 8.3 |
| " | 16 | Betaine | 14.4 | 15.7 | 15.1 | 11.8 |
| " | 20 | " | ..... | ..... | 15.6 | 12.5 |
| " | 24 | " | ..... | ..... | 15.9 | 24.6 |
| Lactalb. 7HAA | 16 | None | 5.9 | 40.6 | 5.6 | 29.1 |
| " " | 20 | " | ..... | ..... | 9.3 | 23.6 |
| " " | 24 | " | ..... | ..... | 14.4 | 18.8 |
| " " | 16 | Choline | 15.7 | 7.4 | 16.2 | 12.3 |
| " " | 20 | " | ..... | ..... | 16.9 | 8.3 |
| " " | 24 | " | ..... | ..... | 18.3 | 6.1 |
| " " | 16 | Betaine | 14.7 | 17.3 | 15.5 | 25.6 |
| " " | 20 | " | ..... | ..... | 17.0 | 11.7 |
| " " | 24 | " | ..... | ..... | 17.3 | 7.6 |
| Lactalb. ME–3 | 16 | None | 4.4 | 20.5 | 6.5 | 32.1 |
| " " | 20 | " | ..... | ..... | 6.0 | 26.9 |
| " " | 24 | " | ..... | ..... | ..... | ..... |
| " " | 16 | Choline | 14.6 | 6.6 | 15.7 | 6.4 |
| " " | 20 | " | ..... | ..... | 15.9 | 8.0 |
| " " | 24 | " | ..... | ..... | 17.7 | 6.8 |
| " " | 16 | Betaine | 14.8 | 17.5 | 15.7 | 22.1 |
| " " | 16 | " | 31.8 | 13.0 | ..... | ..... |
| " " | 20 | " | ..... | ..... | 16.0 | 21.4 |
| " " | 24 | " | ..... | ..... | 17.1 | 15.1 |
| " " | 16 | Dimethylaminoethanol | 11.2* | 9.9 | ..... | ..... |
| " " | 16 | " | 22.4† | 5.8 | ..... | ..... |

* Includes 6.8 mg. dimethylaminoethanol.
† Includes 20.4 mg. dimethylaminoethanol.

among the choline- or betaine-supplemented groups during the first 12-day critical period. This objective evidence would seem to indicate that betaine and choline are equally effective in supplying labile methyl. However, such

an unqualified conclusion is not wholly justified as will be noted from the records in table 1. Under comparable conditions involving the qualitative character and quantitative intake of the protein, betaine exerts substantially less lipotropic action than the same amount of choline. A two-fold increase in the betaine supplement furnished with the lactalbumin ME–3 at the 16 per cent level, did not significantly increase the lipotropic effect. Dimethylaminoethanol supplementing the same protein was substantially as effective in lipotropic action as choline. The percentage of choline in the liver lipids from the betaine- and choline-supplemented groups did not vary significantly, the general average being 0.162 per cent. The choline level of the liver lipids from the animals receiving the higher amount of dimethylaminoethanol was consistently higher, averaging 0.22 per cent.

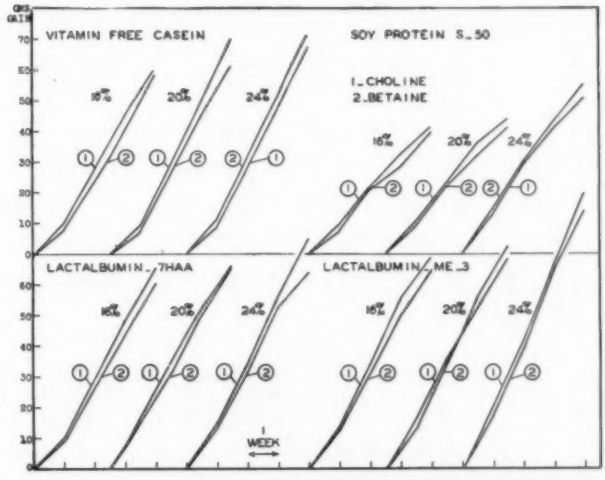



FIG. 1. Comparative growth-promoting value of choline and betaine supplementing milk proteins and soy protein.

Notwithstanding the lower lipotropic action of betaine compared with choline, uric acid retention in the kidneys was uniformally as low or lower than that found in the kidneys of the choline-supplemented animals (tables 2 and 3). The riboflavin concentrations in the kidneys at the critical 8-day period were also at a normal level, comparable with those found in the animals receiving adequate choline supplements (table 2).

In order to determine the lipotropic action and effect upon uric acid retention of betaines other than the betaine of glycine, a mixture of betaines corresponding to the amino acids of lactalbumin 7HAA was prepared for test purposes as follows:

The protein was hydrolyzed with HCl and the humin and the greater part of the acid removed. The solution was made very slightly alkaline and

TABLE 2

*The comparative merits of choline, betaine and dimethylaminoethanol for maintaining normal uric acid and riboflavin levels in the kidneys of animals receiving a 16% protein diet*

| Dietary protein | Supplement | After 8 days | | | | | After 28 days | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Choline equivalent | Uric acid in kidneys | Riboflavin in kidneys (γ/gm.) | | | Choline equivalent | Uric acid in kidneys | Riboflavin in kidneys (γ/gm.) | | |
| | | | | "Free" | "Bound" | "Total" | | | "Free" | "Bound" | "Total" |
| | | *mg./day* | *mg.* | | | | *mg./day* | *mg.* | | | |
| V. F. casein | None | 10.1 | 0.014 | ..... | ..... | ..... | 9.5 | 0.018 | 3.9 | 16.2 | 20.1 |
| " " | Choline | 14.3 | 0.011 | ..... | ..... | ..... | 15.7 | 0.016 | 3.7 | 20.4 | 24.1 |
| " " | Betaine | 14.5 | ..... | ..... | ..... | ..... | 15.5 | 0.014 | 3.7 | 20.3 | 24.0 |
| Soy S–50 | None | 3.2 | 0.027 | 2.3 | 14.3 | 16.6 | 3.6 | 0.022 | 3.1 | 10.0 | 13.1 |
| " " | Choline | 14.2 | 0.017 | 3.3 | 22.3 | 25.6 | 15.1 | 0.017 | 3.8 | 15.5 | 19.3 |
| " " | Betaine | 14.4 | 0.016 | 3.4 | 20.5 | 23.9 | 15.1 | 0.016 | 3.8 | 15.4 | 19.2 |
| Lactalb. 7HAA | None | 5.9 | 0.035 | ..... | ..... | ..... | 5.6 | 0.020 | 2.6 | 16.7 | 19.3 |
| " " | Choline | 15.7 | 0.017 | ..... | ..... | ..... | 16.2 | 0.017 | 3.9 | 15.9 | 19.8 |
| " " | Betaine | 14.7 | ..... | ..... | ..... | ..... | 15.5 | 0.013 | 4.1 | 16.3 | 20.7 |
| Lactalb. ME–3 | None | ..... | ..... | ..... | ..... | ..... | 6.5 | 0.015 | 3.9 | 14.6 | 18.5 |
| " " | Choline | ..... | ..... | ..... | ..... | ..... | 15.7 | 0.015 | 4.9 | 18.5 | 23.4 |
| " " | Betaine | 14.8 | 0.012 | 2.6 | 15.8 | 18.4 | 15.7 | 0.014 | 5.0 | 18.3 | 23.3 |
| " " | " | 31.8 | 0.010 | 2.8 | 22.2 | 25.0 | ..... | ..... | ..... | ..... | ..... |
| " " | Dimethylaminoethanol | 11.2* | 0.010 | 2.7 | 19.1 | 21.8 | ..... | ..... | ..... | ..... | ..... |
| " " | " | 22.4† | 0.010 | 2.9 | 23.9 | 26.8 | ..... | ..... | ..... | ..... | ..... |

* Includes 6.8 mg. dimethylaminoethanol.
† Includes 20.4 dimethylaminoethanol.

concentrated in vacuum to remove ammonia. The methylation was performed essentially in the least drastic manner described by Carter and Melville (3). Four moles of methyl sulfate and four moles of sodium hydroxide per atom of nitrogen were used. Cooling and slow additions of the reagents were employed to avoid formation of toxic tetramethyl ammonium salts (11)

TABLE 3

*The comparable effectiveness of choline and betaine in maintaining low uric acid retention in the kidneys on 16% protein diets*

| Protein | Time | Choline equivalent intake per day | | | | Liver fat | Uric acid in kidneys |
|---|---|---|---|---|---|---|---|
| | | As methionine in protein | Supplement | Amt. | Total | | |
| | *days* | *mg.* | | *mg.* | *mg.* | *%* | *mg.* |
| Soy protein S–50 | 8 | 3.1 | None | ..... | 3.1 | 19.3 | 0.024 |
| " " " | " | 3.3 | " | ..... | 3.3 | 22.3 | 0.031 |
| " " " | " | 3.2 | " | ..... | 3.2 | 22.8 | 0.027 |
| " " " | " | 3.3 | " | ..... | 3.3 | 24.3 | 0.025 |
| Soy protein S–50 | 12 | 3.6 | None | ..... | 3.6 | 24.5 | 0.033 |
| " " " | " | 3.3 | " | ..... | 3.3 | 25.4 | 0.031 |
| " " " | " | 3.5 | " | ..... | 3.5 | 22.9 | 0.036 |
| Soy protein S–50 | 8 | 3.9 | Choline | 10.5 | 14.4 | 5.2 | 0.017 |
| " " " | " | 3.7 | " | " | 14.2 | 6.6 | 0.019 |
| " " " | " | 2.9 | " | " | 13.4 | 4.5 | 0.019 |
| " " " | " | 3.8 | " | " | 14.3 | 6.1 | 0.016 |
| Soy protein S–50 | 12 | 4.3 | Choline | 10.5 | 14.8 | 7.2 | 0.017 |
| " " " | " | 3.4 | " | " | 13.9 | 7.0 | 0.014 |
| " " " | " | 3.4 | " | " | 13.9 | 6.5 | 0.013 |
| " " " | " | 4.2 | " | " | 14.7 | 8.0 | 0.014 |
| Soy protein S–50 | 8 | 4.1 | Betaine | 10.5 | 14.6 | 20.9 | 0.012 |
| " " " | " | 4.0 | " | 10.5 | 14.9 | 22.2 | 0.013 |
| " " " | " | 3.5 | " | 10.5 | 14.0 | 20.8 | 0.012 |
| " " " | " | 3.8 | " | 10.5 | 14.5 | 19.7 | 0.011 |
| Soy protein S–50 | 12 | 4.2 | Betaine | 10.5 | 14.7 | 16.7 | 0.013 |
| " " " | " | 3.9 | " | " | 14.4 | 16.4 | 0.014 |
| " " " | " | 4.4 | " | " | 14.9 | 18.4 | 0.012 |
| " " " | " | 3.6 | " | " | 14.1 | 17.7 | 0.013 |
| Lactalbumin ME–3 | 8 | 5.4 | Betaine | 8.8 | 14.2 | 24.1 | 0.009 |
| " " | " | 5.5 | " | " | 14.3 | 22.0 | 0.014 |
| " " | " | 5.9 | " | " | 14.7 | 23.3 | 0.013 |
| " " | " | 5.3 | " | 26.8 | 32.1 | 15.1 | 0.008 |
| " " | " | 4.5 | " | " | 31.3 | 19.0 | 0.008 |
| " " | " | 5.8 | " | " | 32.6 | 13.2 | 0.011 |
| " " | " | 4.3 | " | " | 31.1 | 14.7 | 0.012 |

which otherwise might result from the further methylation of any trimethylamine split off from certain unstable betaines (6). After refluxing the mixture with HCl, sulfate was removed, and the solution neutralized and evaporated in vacuum. The betaines were taken up in ethanol and filtered from the greater part of the sodium chloride. The final product was an aqueous concentrate of the mixed betaines. From the percentage of total nitrogen in the hydrolysate and that in the methylated product precipitable by phos-

photungstic acid, it was estimated that conversion of the mono-amino acid and presumably of the total amino acids, was 80 per cent complete. Considering the relative amounts of the amino acids in the starting material, it appeared that 67 per cent of the nitrogen was in the form of $\alpha$ betaine groups. A qualitative test showed no more than a trace of tetramethyl ammonium salts. The final product obviously consisted largely of betaines other than those of glycine, alanine and cystine, the only betaines thus far reported to be lipotropic (13).

The mixed betaines prepared from the lactalbumin supplementing the 16 per cent soy protein diet showed no significant lipotropic effect. The increase in retention of uric acid and reduction in the riboflavin concentration in the kidneys were of the same order of magnitude as found in the unsupplemented controls for the same protein (tables 1 to 4).

The relatively greater lipotropic effect of choline compared with betaine lends support to the theory that the intact choline molecule is involved in

TABLE 4

*The negative effect of mixed betaines in preventing the hepatico-renal syndrome when furnished as a 10.5 mg. (calculated as glycine betaine) supplement with 16% soy protein*

| Time | Liver fat | Uric acid in kidneys | Riboflavin in kidneys (γ/gm.) | | |
|---|---|---|---|---|---|
| | | | "Free" | "Bound" | Total |
| | % | mg. | | | |
| After 8 days | 21.2 | 0.027 | 1.8 | 15.9 | 17.7 |
| After 12 days | 23.1 | 0.028 | 1.9 | 10.7 | 12.6 |
| After 28 days | 31.4 | 0.022 | 2.9 | 9.3 | 12.2 |

lipotropic action. (Arseno-choline which is apparently not a methyl donor is lipotropic (19). If this is true, the lipotropic action of betaine or its labile methyl must be involved in the bio-synthesis of choline in an indirect manner thus accounting for its comparatively less effectiveness.

Dimethylaminoethanol, suggested by duVigneaud (8) as worthy of trial proved to have a high lipotropic action substantially equivalent to that of choline. It was also found that the choline concentration of the liver lipids of the animals receiving this supplement were consistently and substantially higher than from the animals receiving either the betaine or choline supplements. Theoretically, this compound should have at least a transitory existence during the biological synthesis and degradation of choline. It would be unwarranted however, to assume that this dimethyl compound *per se* necessarily gives up labile methyl when an alternate explanation of its beneficial effect on the labile methyl economy is conceivable, namely, the fact that its conversion to choline requires the addition of only one methyl group, whereas, the conversion of aminoethanol to choline would require the addition of three methyl groups.

The data presented in this and the previous paper (16) have concerned the essentiality of choline or labile methyl in the dietary of young animals. The evidence indicates a spontaneous correction of choline deficiency among the survivors after a critical two-week period, as determined by improvement in renal function and resumption of growth; a parallel decrease in liver fat levels in the absence of choline supplement has not been apparent in these studies through a 28-day observation period. In order to determine the influence of labile methyl deficiency on older animals, groups of weanling rats were provided with the usual dietary regimen containing lactalbumin

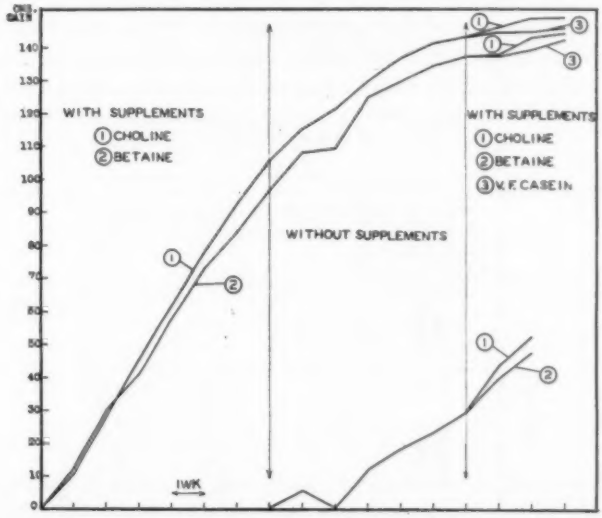



FIG. 2. Influence of choline and betaine and absence of labile methyl supplements on growth of young and mature rats.

ME–3 at the 16 per cent level. One-third of the animals received no labile methyl supplement; another third received a pre-calculated choline supplement designed to provide a daily choline equivalent intake of 13 to 15 mg.; and the remaining groups received a betaine supplement in the same amount as furnished the choline-supplemented animals. The supplements were continued for seven weeks and then withdrawn for six weeks, followed by supplementation with choline or change of the dietary protein from lactalbumin to casein for a three-week period. The results from this series of comparisons involving alternate dietary adequacy and impoverishment of choline and betaine are shown in figure 2 and table 5.

There was substantially no difference in growth rate between the choline- and betaine-supplemented animals; the growth pattern of the surviving unsupplemented animals was substantially less than from those receiving

TABLE 5

*The relative influence of choline and betaine and no labile methyl supplements on the liver fat level and kidney function of mature rats*

| Supplement | Time period | Choline equiv. intake | Supplement | Time period | Choline equiv. intake | Supplement | Time period | Choline equiv. intake | Liver fat | Uric acid in kidneys | Riboflavin in kidneys (γ/gm.) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | "Free" | "Bound" | Total |
| | *wks.* | *mg./day* | | *wks.* | *mg/day* | | *wks.* | *mg./day* | % | *mg.* | γ | γ | γ |
| None | 7 | 3.8 | ..... | ..... | ..... | ..... | ..... | ..... | 33.7 | 0.015 | 3.4 | 16.2 | 19.6 |
| None | 7 | 3.8 | Choline | 2 | 14.9 | ..... | ..... | ..... | 29.4 | 0.012 | 6.7 | 15.6 | 22.3 |
| None | 7 | 3.8 | Betaine | 2 | 14.2 | ..... | ..... | ..... | 27.9 | 0.013 | 6.0 | 19.3 | 25.3 |
| Choline | 7 | 15.3 | ..... | ..... | ..... | ..... | ..... | ..... | 7.8 | 0.016 | 6.0 | 18.4 | 24.5 |
| Choline | 7 | 15.3 | None | 2 | 7.6 | ..... | ..... | ..... | 10.7 | 0.013 | 6.0 | 14.5 | 20.5 |
| Choline | 7 | 15.3 | None | 6 | 7.7 | ..... | ..... | ..... | 19.4 | ..... | 6.2 | 17.5 | 23.7 |
| Choline | 7 | 15.3 | None | 6 | 7.7 | Choline | 3 | 18.2 | 11.1 | ..... | ..... | ..... | ..... |
| Choline | 7 | 15.3 | None | 6 | 7.7 | V. F. casein* | 3 | 12.5 | 12.1 | ..... | ..... | ..... | ..... |
| Betaine | 7 | 15.4 | ..... | ..... | ..... | ..... | ..... | ..... | 24.1 | 0.018 | 5.5 | 16.7 | 22.5 |
| Betaine | 7 | 15.4 | None | 2 | 7.2 | ..... | ..... | ..... | 23.2 | 0.013 | 6.6 | 18.1 | 24.7 |
| Betaine | 7 | 15.4 | None | 6 | 7.4 | ..... | ..... | ..... | 22.4 | ..... | 6.9 | 16.1 | 23.0 |
| Betaine | 7 | 15.4 | None | 6 | 7.4 | Choline | 3 | 17.3 | 10.7 | ..... | ..... | ..... | ..... |
| Betaine | 7 | 15.4 | None | 6 | 7.4 | V. F. casein* | 3 | 11.1 | 16.2 | ..... | ..... | ..... | ..... |

* Vitamin-free casein substituted in lieu of Lactalbumin 116–ME–3.

either of the supplements. Choline and betaine supplied for two weeks following the 7-week period of deficiency brought about a somewhat improved growth rate; liver fat levels, however, decreased only a minor degree. Kidney function was distinctly improved as judged by decrease in uric acid retention and increase in riboflavin concentration of the kidney tissue.

The withdrawal of choline for a period of six weeks following a 7-week supplementation period caused an elevation in liver fat levels, averaging 19.4 per cent as compared with an average of 7.8 per cent at the time the choline was discontinued. Evidence of suppressed kidney function was not pronounced. No significant change in growth rate occurred following the withdrawal of the choline. The elimination of the betaine supplement after 7 weeks caused no significant change in liver fat level, the values remaining relatively high at the same magnitude as prevailed during the initial period of supplementation. A distinct cessation in growth rate occurred the second

TABLE 6

*The negative effect of glycine in preventing renal damage resulting from choline deficiency with a 16% lactalbumin dietary*

(Observations after 8 days)

| Glycine supplement | Ave. kidney weight | Ave. liver fat | Uric acid in kidneys retained | Riboflavin in kidneys (γ/gm.) | | | Wt. gain |
|---|---|---|---|---|---|---|---|
| | | | | "Free" | "Bound" | Total | |
| *mg.* | *gm.* | *%* | *mg.* | | | | *gm.* |
| 17.1 | 1.240 | 15.6 | 0.023 | 2.1 | 11.9 | 14.0 | 2(loss) |
| 4.2 | 1.290 | 17.7 | 0.021 | 2.0 | 9.1 | 11.1 | 2(loss) |

week following withdrawal of the betaine, but resumed at the previous rate hereafter; no evidence of regression in renal function was apparent.

A resumption of the choline supplement following the six-week period of dietary deficiency brought about a decrease in liver fat levels and a temporary stimulation in growth rate. Likewise, the substitution of casein as the dietary protein in lieu of the lactalbumin caused a drop in liver fat concentration nearly equal to that caused by choline; no significant stimulation of growth was observed.

The contrasting lipotropic action of choline and betaine in conjunction with substantially equivalent growth-promoting properties and equal or greater effectiveness of betaine in maintaining normal kidney function suggests a plurality of causative factors contributing to the hepatico-renal syndrome. Since the betaine of glycine is effective in maintaining normal renal function, whereas the mixed betaines of other amino acids was not, the question of glycine inadequacy as a factor contributing to the renal pathology in young rats has been considered. Lack of glycine has been shown to affect adversely glomerular circulation (2, 17) and it has also been shown that an

increase in elimination of uric acid in man results from the ingestion of glycine (5, 10). It has been postulated that glycine is essential for growth but since the body has a large potential capacity for its synthesis (presumably in the liver) growth requirements are readily met by normal metabolism (15). In order to determine if glycine would prevent the characteristic renal damage resulting from choline deficiency, glycine supplements were provided with the usual basal dietary and regimen, using lactalbumin ME–3 at the 16 per cent level in the absence of choline or betaine. No beneficial results were obtained under the conditions prevailing in these tests (table 6).

## SUMMARY

1. Betaine supplementation of low fat diets containing casein, lactalbumin or soy protein was substantially as effective as the same amount of choline in promoting growth in young rats, in the prevention of hemorrhagic degeneration of the kidneys, and in maintaining normal kidney functions; betaine exerted a distinctly less lipotropic action.

2. The amount of choline in the liver lipids of animals receiving choline and betaine supplements was substantially the same, averaging about 0.16 per cent.

3. Dimethylaminoethanol as a supplement to a diet containing lactalbumin showed a marked lipotropic action substantially equal to that of choline.

4. A mixture of betaines consisting largely of betaines other than those of glycine, alanine and cystine prepared by the methylation of the amino acids derived from lactalbumin was of no value in preventing the hepatico-renal syndrome resulting from choline deficiency.

5. Choline or betaine supplements are seemingly less essential for mature rats than for young animals.

6. Choline impoverishment of the dietary of mature animals is reflected by an increase in the percentage of fat in the liver; the magnitude of the effect of the absence of choline from the dietary of mature rats on kidney function is not as great as occurs in young animals.

7. Glycine supplementation of the diet of young rats is not effective in preventing the characteristic renal syndrome resulting from choline deficiency.

## REFERENCES

(1) Best, C. H., and Huntsman, M. E. Jour. Physiol., **75**: 405. 1932.
(2) Brühl, H. Arch. f. die Gesam. Physiol., **220**: 380. 1928.
(3) Carter, H. E., and Melville, D. B. Jour. Biol. Chem., **133**: 109. 1940.
(4) Chandler, J. P., and du Vigneaud, V. Jour. Biol. Chem., **135**: 223. 1940.
(5) Christman, A. A., and Mosier, E. C. Jour. Biol. Chem., **83**: 11. 1929.
(6) Dakin, H. D. Jour. Biol. Chem., **140**: 847. 1940.
(7) du Vigneaud, V., Chandler, J. P., Moyer, A. W., and Keppel, D. M. Jour. Biol. Chem., **131**: 57. 1939.

(8) DU VIGNEAUD, V., CHANDLER, J. P., MOYER, A. W., AND KEPPEL, D. M. Jour. Biol. Chem., **131**: 73. 1939.
(9) GRIFFITH, W. H., AND MULFORD, D. J. Amer. Chem. Soc. Jour., **63**: 929. 1941.
(10) LEWIS, H. B., DUNN, M. S., AND DOISY, E. A. Jour. Biol. Chem., **36**: 9. 1918.
(11) MAWSON, E. H., AND WELCH, A. DEM. Biochem. Jour., **30**: 417. 1936.
(12) MCGINNIS, J., NORRIS, L. C., AND HEUSER, G. F. Soc. Expt. Biol. and Med. Proc., **51**: 293. 1942.
(13) MOYER, A. W., AND DU VIGNEAUD, V. Jour. Biol. Chem., **143**: 373. 1942.
(14) PLATT, A. P. Biochem. Jour., **33**: 505. 1939.
(15) QUICK, A. J. Jour. Biol. Chem., **92**: 65. 1931.
(16) SUPPLEE, G. C., GALL, L. S., AND CAUL, J. F. JOUR. DAIRY SCI. **28**: 435. 1945.
(17) WATZADSE, G. Arch. f. die Gesam. Physiol., **219**: 694. 1928.
(18) WEBSTER, G. T. Jour. Clin. Invest., **21**: 385. 1942.
(19) WELCH, A. D., AND LANDAU, R. L. Jour. Biol. Chem., **144**: 581. 1942.

# THE USE OF SIMPLIFIED DIETS IN THE STUDY OF THE FAT METABOLISM OF THE MAMMARY GLAND[1]

O. W. KAUFMANN[2] AND J. C. SHAW

*Department of Dairy Industry, Storrs Agricultural Experiment Station, Storrs, Connecticut*

In recent years the origin of the short-chain fatty acids of milk fat has been the subject of much discussion. The occurrence of these lower fatty acids in milk fat, but not in blood fat in detectable quantities, has given rise to many theories as to their origin. The two main theories are: 1) the lower fatty acids are formed in the catabolism of the long-chain fatty acids, and 2) the lower fatty acids are formed from carbohydrate.

A new approach to the problem regarding the origin of these lower fatty acids was made in this work. A study of the milk fat constants of cows on basic diets was undertaken with the hope of finding a diet which would decrease the short-chain fatty acids and yet supply sufficient energy for maintenance and milk production. Should such a diet be found, various possible precursor substances could then be added and the effect upon the lower fatty acids noted. However, should such a finding prove impossible in this particular study, some information relative to the carbohydrate theory of origin might be obtained. The basis for this particular approach to the problem is the finding of Eckles and Palmer (2) that the short-chain fatty acids of milk fat are lowered markedly by inanition, and the finding of Shaw *et al.* (4) that marked hypoglycemia does not decrease the lower fatty acids of the milk fat of cows with ketosis, as long as the appetite is not impaired appreciably. This suggests that the decrease in the lower fatty acids produced by inanition is not caused by a depletion of the carbohydrate available to the lactating gland. It was the purpose of this study to obtain additional information concerning the relationship between food intake and synthesis of the lower fatty acids of milk fat by the cow.

## METHODS

In these experiments the cows had been on a standard diet for several months before being placed on the special diets. In order to make certain that the milk-fat constants were not affected by inanition, all of the feed received by the cows was weighed during a seven-day preliminary period. During this period the experimental cows received from 14 to 16 pounds of hay, 15 pounds of silage and sufficient concentrate to maintain a caloric

Received for publication December 9, 1944.

[1] This investigation was supported in part by a grant from the Chas. M. Cox Co.

[2] The experimental data in this paper are taken from a thesis presented by O. W. Kaufmann in partial fulfillment of the requirements for the degree of Master of Science in Dairy Industry, University of Connecticut.

intake somewhat in excess of the actual requirements (Haecker). This will be referred to as the basal diet. A composite of the morning and evening milk was taken at the beginning of the experimental period and at varying intervals thereafter for the determination of the iodine number and the Reichert-Meissl value of the milk fat.

The iodine numbers (Hanus) and the Reichert-Meissl values were determined according to the methods outlined in the Official and Tentative Methods of Analysis (1). The method used for blood glucose was that recommended by Shaffer and Somogyi (3) as modified by Somogyi (5, 6).

The simplified diets were mixed with water and either pumped directly into the rumen by means of a stomach tube and pump or placed in the rumen through a rumen fistula. In all cases, the object was to feed a sufficient amount of such diets to exceed slightly the caloric requirements for maintenance and milk production. The minimum period of time for such feeding was set at 24 hours, with the optimum at 48 hours. In those cases in which no signs of distress were noted, the experiment was continued for a longer period. Short feeding periods were considered adequate for the purposes of this study because the most marked changes in the Reichert-Meissl value and iodine number occur during the first 48 hours of fasting.

## RESULTS

Diets made up solely of skim milk, skim milk plus skim milk powder, skim milk plus added fat and glucose, starch, starch plus glucose, and casein were force-fed by means of a stomach tube. In one case, skim milk and

TABLE 1

*The effect of inanition upon the Reichert-Meissl value and iodine number of milk fat*

| Cow number | Feeding schedule | Hours after last feeding | Milk production, lbs. | Milk-fat constants | |
|---|---|---|---|---|---|
| | | | | Reichert-Meissl value | Iodine number |
| D.E. 103 | Basal diet AM | ..... | 9.3 | 22.96 | 40.71 |
| | Basal diet PM | ..... | 7.2 | | |
| | No feed AM | 12 | 9.0 | ..... | ..... |
| | No feed PM | 24 | 6.9 | 18.73 | 46.00 |
| | No feed AM | 36 | 4.8 | | |
| | No feed PM | 48 | 3.6 | 14.46 | 47.28 |
| | No feed AM | 60 | 2.8 | | |
| | No feed PM | 72 | 2.0 | 13.01 | 47.25 |
| | No feed AM | 84 | 2.4 | | |
| D.E. 176* | Basal diet AM | ..... | 15.8 | 28.01 | 39.65 |
| | Basal diet PM | ..... | 13.2 | | |
| | No feed AM | 12 | 15.7 | ..... | ..... |
| | No feed PM | 24 | 11.4 | 21.34 | 46.26 |
| | No feed AM | 36 | 9.8 | 18.56 | 47.00 |
| | No feed PM | 48 | 7.5 | 17.37 | 45.77 |

* Blood glucose at the beginning, 49.06 mg. per cent; after 48 hours of fasting, 35.52 mg. per cent.

casein were fed through a rumen fistula. All liquid diets were warmed to 37° C. before feeding.

*Inanition.* Two cows, D.E. 103 and D.E. 176, were fasted for periods of 72 and 48 hours respectively. The change in milk production and in the milk fat constants are shown in table 1. It will be noted that the changes in the Reichert-Meissl values and iodine numbers were marked after 24 to 36 hours of fasting. The Reichert-Meissl values are of particular interest as they give comparative indications of the amounts of butyric and hexanoic acids present in the milk fat. The blood glucose of cow No. D.E. 176 decreased from 49.06 mg. per cent to 35.52 mg. per cent after 48 hours of fasting. During this same period the Reichert-Meissl value decreased from 28.01 to 17.37. It has been pointed out before (Shaw *et al.*) that, although

TABLE 2

*The effect of feeding skim milk plus added fat and glucose on the Reichert-Meissl value and the iodine number of milk fat*

| Time of day | Feeding schedule | | | | Milk production, lbs. | Milk fat constants | |
|---|---|---|---|---|---|---|---|
| | Hours on simple diet | Skim milk, lbs. | Milk fat, lbs. | Glucose, lbs. | | R.M. | I.V. |
| AM | Basal diet | ..... | ..... | ..... | 10.7 | 21.84 | 44.32 |
| PM | Basal diet | ..... | ..... | ..... | 7.9 | | |
| AM | ..... | 60 | 1.2 | 2.50 | 9.6 | ..... | ..... |
| PM | 12 | 60 | 1.2 | 2.50 | 7.9 | ..... | ..... |
| AM | 24 | ..... | ..... | ..... | 10.2 | ..... | ..... |
| PM | 36 | 90 | 1.8 | 3.75 | 7.9 | 23.35 | 41.82 |
| AM | 48 | 60 | 1.2 | 2.50 | 10.6 | 20.45 | 42.58 |
| PM | 60 | 60 | 1.2 | 2.50 | 7.5 | ..... | ..... |
| AM | 72 | ..... | ..... | ..... | 10.2 | 39.98 | 33.75 |

cows with ketosis may exhibit a blood glucose value much lower than this, the Reichert-Meissl value is not particularly low unless the cow is "off feed."

*Skim milk plus added fat and glucose.* In the initial experiment, it was desired to feed a diet devoid of cellulose as a check on the possibility that by-products of cellulose digestion in the rumen were precursors of the lower fatty acids of milk. The diet consisted of skim milk to which was added sufficient milk fat and glucose to supply the necessary energy. The cow drank the milk the first day but refused to drink the diet on the following day. As a result, one feeding was missed, after which the milk was pumped into the rumen. Scouring was observed on the second day. Rennin was added to the milk in subsequent feedings, and the feces remained firm thereafter.

It will be noted in table 2 that there was a very large increase in the Reichert-Meissl value and a corresponding decrease in the iodine number between the initial and final samples. The fat constants of the intermediate samples were not altered materially, probably because of the failure to retain the milk diet during this period and the missed feeding on the morning of

the second day. Milk production decreased very little during the experimental period.

*Skim milk and skim milk powder.* Data on two cows which were fed skim milk as the sole diet are recorded in table 3. Cow No. D.E. 165 received 12.5 pounds of dried skim milk in 40 pounds of water twice daily. Cow No. D.E. 175 received, twice daily, 40 pounds of skim milk to which had been added 6 pounds of dried skim milk. In both cases, the caloric intake was slightly in excess of requirements (Haecker). On these diets there was an increase in the Reichert-Meissl value and a corresponding decrease in the iodine number of the milk fat. Cow No. D.E. 175 maintained milk

TABLE 3

*The effect of feeding skim milk and skim milk powder on the Reichert-Meissl value and the idoine number of milk fat*

| Cow number | Time of day | Feeding schedule | | | Milk production, lbs. | Milk fat constants | |
|---|---|---|---|---|---|---|---|
| | | Hours on simple diet | Skim milk, lbs. | Skim milk powder, lbs. | | R.M. | I.V. |
| D.E. 165* | AM | Basal diet | | ..... | 18.6 | 26.81 | 37.99 |
| | PM | Basal diet | | ..... | 15.6 | | |
| | AM | ..... | | 12.5 | 17.0 | ..... | ..... |
| | PM | 12 | | 12.5 | 13.7 | ..... | ..... |
| | AM | 24 | | 12.5 | 18.5 | ..... | ..... |
| | PM | 36 | | 12.5 | 14.5 | ..... | ..... |
| | AM | 48 | | ..... | 13.4 | 27.53 | 32.72 |
| D.E. 175 | PM | Basal diet | ..... | ..... | 11.0 | 24.10 | 39.60 |
| | AM | Basal diet | ..... | ..... | 12.0 | | |
| | PM | ..... | 40 | 6 | 11.4 | ..... | ..... |
| | AM | 12 | 40 | 6 | 11.9 | ..... | ..... |
| | PM | 24 | 40 | 6 | 10.8 | ..... | ..... |
| | AM | 36 | 40 | 6 | 12.5 | 30.13 | 34.97 |
| | PM | 48 | 40 | 6 | 11.5 | ..... | ..... |
| | AM | 60 | ..... | ..... | 12.1 | 28.73 | 35.61 |

* Blood glucose at beginning, 48.59 mg. per cent; after 48 hours, 58.76 mg. per cent. cent.

production, whereas the milk production of cow No. D.E. 165 decreased somewhat. The latter animal scoured considerably during the experiment, whereas the feces of cow No. D.E. 175 remained firm, suggesting that the decrease in milk production was due to the scouring. During the 48 hours cow No. D.E. 165 was on the diet of skim milk powder, the blood glucose increased from a normal value of 48.59 mg. per cent to an abnormally high value of 58.76 mg. per cent.

*Starch and glucose.* An attempt was made to determine what particular skim milk substance or substances were responsible for the increase in the lower fatty acids of milk fat. Data are presented in table 4 on three cows which received diets of cornstarch, cornstarch and glucose, and soluble starch and glucose. In each case, the carbohydrate was added to 40 pounds of warm water before being pumped into the rumen.

The diet of cornstarch and glucose fed to cow No. D.E. 166 maintained milk production very close to normal and resulted in an increased Reichert-Meissl value and a decreased iodine number. The diet of cornstarch received by cow No. D.E. 163 and the diet of soluble starch and glucose received by cow No. D.E. 177 did not prevent either a decrease in the Reichert-Meissl value or an increase in the iodine number. However, the change in these two fat constants was much more marked when cows were subjected to complete fasting over this same period of time. Likewise, the carbohydrate

TABLE 4

*The effect of feeding starch and glucose on the Reichert-Meissl value and the iodine number of milk fat*

| Cow Number | Time of day | Feeding Schedule | | | | Milk Production, lbs. | Milk-fat constants | |
|---|---|---|---|---|---|---|---|---|
| | | Hours on simple diet | Corn starch, lbs. | Glucose, lbs. | Soluble starch, lbs. | | R.M. | I.V. |
| D.E. 163* | AM | Basal diet | ..... | ..... | ..... | 17.0 | 26.53 | 37.91 |
| | PM | Basal diet | ..... | ..... | ..... | 14.0 | ..... | ..... |
| | AM | ..... | 10 | ..... | ..... | 15.8 | ..... | ..... |
| | PM | 12 | 10 | ..... | ..... | 13.2 | ..... | ..... |
| | AM | 24 | 10 | ..... | ..... | 15.7 | ..... | ..... |
| | PM | 36 | 14 | ..... | ..... | 11.4 | ..... | ..... |
| | AM | 48 | ..... | ..... | ..... | 9.8 | 22.21 | 41.69 |
| D.E. 166† | AM | Basal diet | ..... | ..... | ..... | 8.3 | 25.26 | 36.26 |
| | PM | Basal diet | ..... | ..... | ..... | 6.3 | | |
| | AM | ..... | 3.25 | 3.25 | ..... | 8.8 | ..... | ..... |
| | PM | 12 | 3.25 | 3.25 | ..... | 6.3 | ..... | ..... |
| | AM | 24 | 3.25 | 3.25 | ..... | 8.4 | ..... | ..... |
| | PM | 36 | 3.25 | 3.25 | ..... | 6.1 | ..... | ..... |
| | AM | 48 | ..... | ..... | ..... | 8.1 | 28.55 | 33.21 |
| D.E. 177 | PM | Basal diet | | ..... | ..... | 12.5 | 26.09 | 36.41 |
| | AM | Basal diet | | ..... | ..... | 17.9 | ..... | ..... |
| | PM | ..... | | 5 | 4 | 12.9 | ..... | ..... |
| | AM | 12 | | 5 | 4 | 17.4 | ..... | ..... |
| | PM | 24 | | 5 | 4 | 12.9 | ..... | ..... |
| | AM | 36 | | 5 | 4 | 10.1 | 23.71 | 39.22 |

* Blood glucose at beginning, 50.17 mg. per cent; after 48 hours on starch diet, 59.67 mg. per cent.

† Blood glucose after 48 hours on starch-glucose diet, 58.31 mg. per cent.

diets prevented the rapid decrease in milk production which occurred during similar periods of fasting, as shown in table 1.

In each of the three experiments, the cows exhibited discomfort by the end of 48 hours. Cow No. D.E. 166, on the cornstarch-glucose diet, exhibited the least signs of digestive disturbance. This may account for the better maintenance of production and for the increased synthesis of the lower fatty acids of milk fat. Soluble cornstarch was used in the case of cow No. D.E. 177 in an unsuccessful attempt to avoid the digestive disturbances noted previously on diets of cornstarch. Indigestion resulted in an increase in blood glucose in those experiments in which blood glucose was determined.

*Casein.* To determine if protein could serve as a dietary precursor of the lower fatty acids of milk fat, three cows were fed casein as the only source of energy. Four to six pounds of casein were soaked in 20 pounds of water for 24 hours. Additional water was then added to facilitate the passage of the mixture through the stomach tube and pump. In the initial trial, the casein was not neutralized. In the next two trials, the casein was neutralized to a pH of approximately 6.8. A severe reaction resulted in all cases within 4 to 8 hours. Death resulted in one case. The other two animals were saved by pumping approximately 10 gallons of warm milk into the rumen. The apparent indigestion may have been due to the physical nature of the casein. Hydrolysates of casein will be used in a continuation of this work.

## SUMMARY

The data show that carbohydrate, when fed as the sole diet, provides the precursors for the synthesis of the lower fatty acids of milk fat. As blood glucose does not appear to be the blood precursor of the lower acids, the dietary carbohydrate must act indirectly. Possibly, the carbohydrate is converted into the necessary precursors in the rumen by the action of certain micro-organisms. It is also possible that it may exert a sparing action on the utilization by other body tissues of substances which act as precursors of the lower fatty acids of milk fat.

## REFERENCES

(1) Association of Official Agricultural Chemists. Official and Tentative Methods of Analysis. 5th Ed. Washington, D. C. 1940.

(2) Eckles, C. H., and Palmer, L. S. The Influence of the Plane of Nutrition of the Cow upon the Composition and Properties of Milk and Butter Fat. Influence of Underfeeding. Mo. Agr. Expt. Sta. Bul. 25. 1916.

(3) Shaffer, P. A., and Somogyi, M. Copper-iodometric Reagents for Sugar Determinations. Jour. Biol. Chem., **100**: 695–713. 1933.

(4) Shaw, J. C., Powell, R. C., Jr., and Knodt, C. B. The Fat Metabolism of the Mammary Gland of the Normal Cow and of the Cow in Ketosis. Jour. Dairy Sci., **25**: 909–921. 1942.

(5) Somogyi, M. A Method for the Preparation of Blood Filtrates for the Determination of Sugar. Jour. Biol. Chem., **86**: 655–663. 1930.

(6) Somogyi, M. A Reagent for the Copper-iodometric Determination of Very Small Amounts of Sugar. Jour. Biol. Chem., **119**: 771–776. 1937.

# THE VITAMIN A POTENCY OF CREAMERY BUTTER PRODUCED IN MINNESOTA[1]

ROBERT JENNESS AND L. S. PALMER[2]
*Division of Agricultural Biochemistry,*
*University of Minnesota, St. Paul, Minnesota*

It is well known that the vitamin A potency of butterfat and butter depends primarily on the carotene content of the cow's ration (2, 3, 15, 16, 17, 18, 20, 21, 26, 28, 29, 31, 32, 33, 37) unless concentrates containing vitamin A itself are fed (1, 10, 11, 12, 14, 18, 22, 34). When no vitamin A *per se* is fed, the distribution of the total potency of the butter between carotene and vitamin A, depending on the animal's ability to convert the former to the latter, is a breed characteristic (3, 7, 9, 19, 29, 35, 38). Logical application of these fundamental factors to production of butter of adequate potency for human nutrition demands, as a prerequisite, a knowledge of the potency of butter produced under present conditions.

The survey reported herein represents an attempt to establish the extent to which butter produced in Minnesota may be relied upon as a source of this vitamin. This survey, constituting Minnesota's share in the national Cooperative Project for the Determination of the Vitamin A Potency of Butter, which was instigated in 1941 by the Committee on Food and Nutrition of the National Research Council, involved the determination of the seasonal fluctuations and geographical differences in the vitamin A potency of Minnesota butter throughout 1943 and the first six months of 1944.

Similar surveys are being conducted in connection with this national project in most of the other important butter-producing states, but to date the only published results are those of Berl and Peterson (6) who have reported averages of 10,500, 9,500, 17,700, and 18,000 I.U. per pound for the total vitamin A potency of butters collected from Wisconsin creameries in January, March, July, and September respectively.

In an independent survey (23), the vitamin A content of sixty-two samples of commercial butters collected in Texas throughout the year was found to average 16,750 I.U. per pound. Such seasonal differences as were noted in this study could not be interpreted since the samples were not necessarily produced in the month in which they were purchased.

Earlier, rather limited studies reported by Booth *et al.* (8), Morgan and Pritchard (30), Tainsh and Wilkinson (36), and Wilkinson (39) indicated that under northern feeding conditions, the content of both carotene and vitamin A in butter rises abruptly to a peak in early summer, declines

Received for publication December 11, 1944.

[1] Paper No. 2203, Scientific Journal Series, Minn. Agr. Expt. Sta., St. Paul, Minnesota.

[2] Deceased March 8, 1944.

slightly during later summer, rises again to a second peak in early autumn, and finally declines gradually to rather uniformly low winter values.

A survey (13) of the carotene and vitamin A contents of Wisconsin market milks corroborated the general picture of seasonal fluctuations previously established for butter (8, 30, 36, 39). That a distinctly different situation exists under southern feeding conditions has been shown by Koehn (24) who found, in Alabama milk, maxima in both vitamin A and carotene in August and minima in February.

## COLLECTION OF SAMPLES

Creamery butter is produced in eighty-six of the eighty-seven counties in Minnesota. On the basis of 1940 production figures, taken from the 1941

FIG. 1. Location of regions and of cooperating creameries.

report of the State Department of Agriculture, Dairy and Food, ten regions were established, each composed of a block of adjacent counties, and each producing approximately ten per cent of the state's butter. Four creameries in each region, each located in a different county, agreed to submit samples at weekly intervals during 1943. The selection of creameries was

based on: 1) their producing 10 per cent or more of the butter in their respective counties, and 2) their assurance of continued cooperation. For the most part they were cooperative organizations drawing cream exclusively from surrounding territory. Only one of the forty failed to cooperate throughout 1943. The geographical relations of the several regions as well as the location of the cooperating creameries within them are shown in figure 1. Although the regions were established on the basis of 1940 production, table 1 shows that their relative annual productions did not change appreciably in the period 1940 to 1943 inclusive.

The samples consisted of one-pound prints from the regular make, except for one ten-pound tub from each region each month for storage studies. They were mailed unrefrigerated to the laboratory, the summer samples being shipped in metal containers to avoid leakage.

TABLE 1

*Distribution of Minnesota butter production among regions in 1940–43*

| Region | Per cent of total annual production | | | |
|---|---|---|---|---|
| | 1940 | 1941 | 1942 | 1943 |
| 1 | 9.4 | 9.7 | 10.0 | 10.0 |
| 2 | 8.8 | 9.2 | 9.0 | 9.5 |
| 3 | 10.1 | 10.4 | 10.6 | 10.9 |
| 4 | 10.2 | 10.5 | 10.9 | 11.4 |
| 5 | 10.1 | 9.9 | 9.9 | 9.9 |
| 6 | 10.4 | 10.2 | 10.8 | 11.3 |
| 7 | 9.2 | 8.4 | 8.2 | 7.7 |
| 8 | 10.3 | 11.0 | 10.6 | 8.6 |
| 9 | 10.9 | 10.4 | 9.8 | 10.0 |
| 10 | 10.6 | 10.3 | 10.2 | 10.9 |

Except for a transition period from winter feeding to pasture conditions, when all the samples received were analyzed, analyses were made at biweekly intervals only, those for two creameries in each region being made on the odd-numbered weeks' samples, those for the other two on the even-numbered weeks' samples.

The roughage available in Minnesota during the winter 1942–43 had been rather badly weathered at harvest.[3] For this reason the project was extended through the first six months of 1944 to ascertain whether the low vitamin A potencies observed in butters produced in the winter of 1943 would be duplicated when roughage quality was more nearly normal. For this extended portion of the project, samples were received from one creamery in each of the ten regions at semi-monthly intervals during January, February, March and April and at weekly intervals from May 15 to June 15 inclusive. All of these samples were analyzed.

[3] Based on information obtained from members of the staff of the Division of Dairy Husbandry, University of Minnesota.

## ANALYTICAL METHODS

Analyses were made in duplicate by the modification of the Koehn and Sherman (25) procedure, recommended by the Technical Committee on Vitamin A Researches,[4] with only minor changes. Briefly this method is as follows: A 10-gram sample of unrendered butter is weighed out and refluxed 10 minutes with 5 ml. of saturated aqueous potassium hydroxide and 20 ml. of absolute methanol on a boiling water bath. The saponification mixture is diluted with 40 ml. of distilled water and cooled to room temperature. It is then transferred to a separatory funnel with an additional 40 ml. of water. The unsaponifiable matter is extracted from the soap with a 100-ml. portion and two 50-ml. portions of peroxide-free ethyl ether. The ethyl ether extract is washed free of alkali, dried over anhydrous sodium sulfate and made to volume.

For the vitamin A determination, one-half of the extract is evaporated to dryness under vacuum, and the unsaponifiable residue is taken up in chloroform. To a 1-ml. aliquot of the chloroform solution in a photoelectric colorimeter tube are added 9 ml. of a 22.5 per cent solution of antimony trichloride in purified and redistilled chloroform. The intensity of the blue color that develops is read at 620 mμ at the point of temporary stability. Since treatment of carotene with antimony trichloride yields a product giving some absorption at 620 mμ, a correction must be made for the amount of such carotene antimony trichloride complex. The azo dyes of butters colored with certified butter color are considered to be equivalent to carotene insofar as the absorption of their antimony trichloride complexes at 620 mμ is concerned. The second half of the ether solution of unsaponifiable matter is likewise evaporated under vacuum, but the residue is taken up in Skellysolve B. The Skellysolve B solution is extracted with successive portions of 92 per cent methanol (94 per cent diacetone alcohol if acid or molasses silage is fed) until the extracts are colorless, washed four times with water, dried over anhydrous sodium sulfate and made to volume. Carotene is determined in this solution in a photoelectric colorimeter at 440 mμ.

The only modifications that we made in the recommended procedure were: 1) use of Skellysolve boiling at 40–45° C. instead of Skellysolve B because our standard curve was made with this grade of Skellysolve and because we had a large stock of it, and 2) addition of a drop of acetic anhydride to the chloroform solution of unsaponifiable matter just before

[4] This committee, appointed by the coordinator of the project, Director C. H. Bailey of the Minnesota Agricultural Experiment Station, consisted of L. A. Maynard (Chm.), L. S. Palmer, C. J. Koehn, H. R. Guilbert, F. P. Zscheile, W. H. Peterson, C. A. Cary, and I. L. Hathaway. After collaborative work upon samples of butterfat circulated by Dr. Koehn as referee, a procedure was recommended. Some of the results obtained in the collaborative work together with a comparison of the results obtained by direct spectrophotometry with those secured by use of the antimony trichloride reaction have been published by Zscheile *et al.* (40).

TABLE 2

*Effect of acetic anhydride on the Carr-Price reaction*

| Chloroform solution of | No acetic anhydride added | | 0.02 ml. acetic anhydride added per ml. $CHCl_3$ soln. | |
|---|---|---|---|---|
| | Vitamin A | Appearance | Vitamin A | Appearance |
| | μg. | | μg. | |
| Vitamin A alcohol 1 | 5.14 | Clear blue | 5.10 | Clear blue |
| 2 | 5.92 | " " | 5.82 | " " |
| Unsaponifiable from butterfat 1 | 5.15 | " " | 5.01 | " " |
| 2 | 6.68 | " " | 6.71 | " " |
| Unsaponifiable from butter 1a | 5.10 | Cloudy | 4.20 | Clear blue |
| b | 4.30 | Clear blue | | |
| 2a | 4.92 | Cloudy | 4.31 | Clear blue |
| b | 4.37 | Clear blue | | |
| 3a | 5.20 | Cloudy | 4.50 | Clear blue |
| b | 4.33 | Clear blue | | |

addition of the antimony trichloride reagent to prevent cloudiness which occasionally developed in the Carr-Price reaction. The data recorded in table 2 indicate that the acetic anhydride *per se* has no effect on the reading provided no cloudiness would develop without it.

The colorimetric equipment consisted of a Cenco Photelometer modified to permit use of matched Pyrex tubes as absorption cells. The instrument was calibrated with β-carotene and vitamin A alcohol solutions. The calibration curves were essentially straight lines having slopes indicated by the K values in table 3; these K's were used to calculate the micrograms of caro-

TABLE 3

*Calibration of the Cenco Photelometer (Modified) for vitamin A and carotene analyses*

| Material | Solvent | Range of concentration | Absorption maximum of filter | $K = \frac{\mu g./ml.}{2-\log G}$ |
|---|---|---|---|---|
| | | μg./ml. | mμ | |
| β-Carotene—S.M.A. | Chloroform | 0.2– 2.4[a] | 400 | 6.500 |
| β-Carotene—S.M.A. | Skellysolve | 0.2– 2.4[a] | 400 | 5.375 |
| Vitamin A alcohol, Distillation Products | Chloroform and $SbCl_3$ reagent | 1.0–12.0[b] | 600 | 28.500 |
| | | | | $K = 2\frac{2-\log G}{\mu g./ml.}$ |
| β-Carotene—S.M.A. | Chloroform and $SbCl_3$ reagent | 10.0–50.0[b] | 600 | 0.0025 |

[a] Concentration of final solution.
[b] Concentration of $CHCl_3$ solution before adding $SbCl_3$ reagent.

tene and vitamin A from the observed galvanometer readings. Absorption by the β-carotene antimony trichloride complex is so small that galvanometer readings cannot be obtained in the range of concentration normally encountered in solutions of unsaponifiable matter from butter. The calibration curve was made at higher concentrations and the factor obtained was considered to apply at lower ones. The validity of this factor for the concentrations encountered in this work was verified by analyses of chloroform solutions containing both vitamin A alcohol and β-carotene and by recovery experiments in which both were added to butter.

During the course of the survey, several referee samples of butterfat were analyzed at intervals at this and other stations. Generally good agreement was attained among analyses for carotene but considerable divergence was found among laboratories in analyses for vitamin A. For this reason it was considered desirable to ascertain the extent of recovery of added vitamin A. In a series of 10 determinations on butters in each of which 5 ml. of the methanol used for saponification were replaced with 5 ml. of a methanol solution containing 54.75 μg. of vitamin A alcohol, recoveries averaged 96.4 ± 1.9 per cent. Recoveries of 92.6 and 91.8 per cent were secured when 108.10 μg. of vitamin A alcohol in 10 ml. of methanol were added to each of two referee butterfat samples. These recoveries are in reasonable agreement with the average of 93.3 per cent found by Cary[5] in 26 determinations.

The standard error of the determination of vitamin A was 0.18 μg. and of the carotene determination 0.13 μg. as computed from the results of the duplicate analyses of the 1,019 samples analyzed in 1943. The experimental error of both determinations showed a slight tendency to increase with an increase in the magnitude of the variate. This is shown by low but significant positive correlations between the sums and differences of duplicate determinations; for the vitamin A determination the correlation coefficient was + 0.16 while for carotene it was + 0.39.

## RESULTS

The data for the 1,019 samples analyzed in 1943 have been summarized by regions and by 13 consecutive seasonal periods of four weeks each.[6] The fifth period has been divided into two sub-periods of two weeks each in order to present more clearly the rapid rise in carotene and vitamin A contents that occurred during that period. The distribution of samples among the several periods is given in table 4A. None of the vitamin A analyses has been corrected for incomplete recovery.

*Regional differences.* The mean vitamin A and carotene contents for each region in each seasonal period are presented, together with their standard errors in tables 4B and 4C respectively. The unweighted means for all

[5] Unpublished communication.

[6] The thirteenth period contained 29 days.

TABLE 4A

*Distribution of butter samples secured from ten regions in Minnesota and analyzed during 1943*

| Seasonal period | Period included 1943 | Region | | | | | | | | | | All regions |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | |
| | | Number of samples | | | | | | | | | | |
| 1 | Jan 1–Jan 28 | 9 | 9 | 7 | 7 | 9 | 8 | 8 | 7 | 9 | 8 | 81 |
| 2 | Jan 29–Feb 25 | 8 | 8 | 8 | 7 | 8 | 8 | 8 | 8 | 8 | 8 | 79 |
| 3 | Feb 26–Mar 25 | 8 | 8 | 8 | 7 | 8 | 7 | 8 | 8 | 7 | 8 | 77 |
| 4 | Mar 26–Apr 22 | 9 | 8 | 8 | 6 | 7 | 9 | 8 | 6 | 6 | 10 | 77 |
| 5a | Apr 23–May 6 | 6 | 6 | 7 | 6 | 5 | 6 | 6 | 5 | 5 | 5 | 57 |
| 5b | May 7–May 20 | 8 | 8 | 5 | 5 | 8 | 8 | 8 | 7 | 7 | 8 | 72 |
| 6 | May 21–June 17 | 7 | 8 | 8 | 6 | 7 | 8 | 8 | 8 | 6 | 8 | 74 |
| 7 | June 18–July 15 | 8 | 7 | 7 | 6 | 6 | 6 | 8 | 8 | 7 | 8 | 71 |
| 8 | July 16–Aug 12 | 8 | 8 | 7 | 5 | 7 | 7 | 8 | 8 | 7 | 8 | 73 |
| 9 | Aug 13–Sept 9 | 8 | 8 | 4 | 6 | 8 | 5 | 8 | 6 | 6 | 9 | 68 |
| 10 | Sept 10–Oct 7 | 8 | 8 | 8 | 6 | 8 | 8 | 8 | 6 | 6 | 8 | 74 |
| 11 | Oct 8–Nov 4 | 8 | 8 | 8 | 6 | 8 | 7 | 8 | 5 | 7 | 8 | 73 |
| 12 | Nov 5–Dec 2 | 8 | 8 | 8 | 5 | 8 | 6 | 8 | 6 | 7 | 8 | 72 |
| 13 | Dec 3–Dec 31 | 7 | 8 | 8 | 6 | 7 | 5 | 8 | 8 | 6 | 8 | 71 |
| All periods | | 110 | 110 | 101 | 84 | 104 | 98 | 110 | 96 | 94 | 112 | 1,019 |

TABLE 4B

*Mean vitamin A content of butters produced in ten regions in Minnesota during 1943*

| Seasonal period | Region | | | | | | | | | | All regions |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | |
| | Vitamin A content | | | | | | | | | | |
| | μg./gm. | μg./gm. | μg./gm. | μg./gm. | μg./gm. | μg./gm. | μg./gm. | μg./gm. | μg./gm. | μg./gm. | μg./gm. |
| 1 | 5.22 ± .16* | 4.79 ± .12 | 4.89 ± .08 | 4.89 ± .16 | 4.29 ± .10 | 4.48 ± .16 | 4.55 ± .08 | 4.32 ± .13 | 4.42 ± .11 | 4.92 ± .11 | 4.68 ± .04 |
| 2 | 5.19 ± .06 | 4.59 ± .10 | 4.89 ± .15 | 4.67 ± .09 | 4.29 ± .14 | 4.59 ± .12 | 4.55 ± .15 | 4.14 ± .14 | 4.76 ± .07 | 4.87 ± .11 | 4.65 ± .04 |
| 3 | 4.77 ± .10 | 4.66 ± .10 | 4.39 ± .16 | 4.36 ± .12 | 4.13 ± .16 | 4.12 ± .14 | 4.20 ± .08 | 4.02 ± .12 | 4.17 ± .12 | 4.46 ± .10 | 4.33 ± .04 |
| 4 | 4.66 ± .09 | 5.23 ± .12 | 4.81 ± .18 | 4.80 ± .17 | 4.33 ± .16 | 4.95 ± .20 | 4.54 ± .10 | 4.84 ± .13 | 4.96 ± .09 | 5.30 ± .14 | 4.86 ± .05 |
| 5a | 5.76 ± .20 | 5.53 ± .19 | 5.73 ± .18 | 5.48 ± .28 | 5.08 ± .16 | 6.61 ± .29 | 4.94 ± .19 | 4.65 ± .21 | 4.98 ± .22 | 6.10 ± .34 | 5.51 ± .09 |
| 5b | 7.42 ± .22 | 6.53 ± .25 | 6.78 ± .19 | 6.94 ± .18 | 7.14 ± .27 | 6.76 ± .29 | 6.31 ± .28 | 6.61 ± .17 | 6.14 ± .30 | 7.58 ± .29 | 6.83 ± .09 |
| 6 | 8.36 ± .15 | 6.78 ± .20 | 7.44 ± .12 | 6.80 ± .10 | 7.73 ± .15 | 7.60 ± .23 | 7.77 ± .15 | 7.03 ± .22 | 7.33 ± .24 | 7.49 ± .19 | 7.44 ± .07 |
| 7 | 7.22 ± .27 | 5.61 ± .16 | 7.05 ± .22 | 6.53 ± .19 | 6.96 ± .10 | 7.31 ± .50 | 8.12 ± .32 | 6.38 ± .21 | 6.94 ± .28 | 6.68 ± .16 | 6.88 ± .10 |
| 8 | 7.06 ± .15 | 5.74 ± .15 | 7.31 ± .21 | 6.53 ± .26 | 7.42 ± .22 | 7.86 ± .14 | 8.16 ± .13 | 7.38 ± .13 | 7.77 ± .21 | 7.24 ± .12 | 7.26 ± .08 |
| 9 | 7.41 ± .16 | 6.45 ± .17 | 8.01 ± .27 | 7.82 ± .21 | 8.47 ± .18 | 8.98 ± .26 | 9.35 ± .30 | 7.74 ± .11 | 8.32 ± .14 | 8.22 ± .12 | 8.06 ± .09 |
| 10 | 7.60 ± .16 | 6.82 ± .22 | 8.00 ± .12 | 7.84 ± .13 | 8.34 ± .25 | 8.53 ± .17 | 8.87 ± .12 | 7.67 ± .29 | 8.18 ± .20 | 8.33 ± .17 | 8.03 ± .07 |
| 11 | 7.04 ± .21 | 6.52 ± .16 | 7.38 ± .27 | 7.06 ± .32 | 7.47 ± .12 | 8.14 ± .24 | 8.48 ± .19 | 7.28 ± .43 | 8.64 ± .24 | 8.20 ± .20 | 7.80 ± .09 |
| 12 | 5.80 ± .17 | 5.04 ± .13 | 5.90 ± .16 | 5.54 ± .20 | 5.90 ± .20 | 6.66 ± .38 | 6.27 ± .09 | 5.56 ± .24 | 6.12 ± .24 | 6.98 ± .23 | 5.99 ± .08 |
| 13 | 5.19 ± .19 | 5.14 ± .15 | 5.54 ± .14 | 5.51 ± .13 | 4.78 ± .15 | 5.40 ± .24 | 5.37 ± .20 | 4.66 ± .14 | 5.38 ± .19 | 5.60 ± .18 | 5.25 ± .06 |
| All periods | 6.31 ± .09 | 5.67 ± .07 | 6.22 ± .10 | 5.99 ± .10 | 6.17 ± .12 | 6.49 ± .13 | 6.55 ± .13 | 5.83 ± .11 | 6.24 ± .12 | 6.58 ± .10 | 6.21 ± .03 |

* Standard error.

regions and for all periods are presented in table 5; the standard deviations of the values for each period and region are also shown.

A statistical analysis of the variance of these data revealed that there are highly significant differences among the regional means (all periods). When the data were plotted, it was found that with the exception of region 2, wherein the vitamin A is the lowest and the carotene the highest in the state, the differences between regions are not consistent throughout the year and the interaction thus introduced was found to be statistically significant. Elimination of the data for region 2 reduced the variance markedly, but still the differences among the remaining nine regions in vitamin A are highly significant while those in carotene are significant. Next to region 2, region 8, while not differing consistently, has the lowest average vitamin A and the highest average carotene content. With elimination of the data for both regions 2 and 8, the differences among the remaining eight regions in carotene content were found to be insignificant. Although the differences in vitamin A content among these eight regions are highly significant; they are of insufficient magnitude to be of much practical importance. The difference between means for regions 2 and 8 is not significant for vitamin A while for carotene it is highly significant.

As shown in table 5, the degree of variability in vitamin A content differs rather widely in the various regions; in carotene content, however, the butters of each region, with the exception of regions 2 and 8, exhibit about the same degree of variability. It is interesting to note that the butters from region 2 have a lower and more uniform vitamin A content and a higher and more variable carotene content than those from any other region.

In arriving at means that would best represent the state in each seasonal period, it was considered advisable to weight the regional mean vitamin A and carotene contents by the regional butter productions, inasmuch as the relative butter productions of the several regions varied somewhat from period to period. Since figures on butter production available from the state department of Agriculture, Dairy, and Food are based on monthly reports, it was necessary to compute the regional butter productions in each seasonal period by prorating the monthly productions among the 13 seasonal periods.

Table 6 furnishes a comparison of the vitamin A and carotene contents in butters from region 2 with the weighted mean contents in butters from the remainder of the state and from the state as a whole. It is readily seen from this table and from a comparison of figures 2 and 3, wherein these data are depicted, that butters produced in region 2 were consistently higher in carotene throughout the year and lower in vitamin A in the summer than butters produced in the remainder of the state. The reason for this divergence became apparent when, upon consulting dairy extension officials, it was found that Guernseys represent a greater proportion of the cattle popu-

TABLE 4C

*Mean carotene content of butters produced in ten regions in Minnesota during 1943*

| Seasonal period | Region | | | | | | | | | | All regions |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | |
| | Carotene content | | | | | | | | | | |
| | μg./gm. | μg./gm. | μg./gm. | μg./gm. | μg./gm. | μg./gm. | μg./gm. | μg./gm. | μg./gm. | μg./gm. | μg./gm. |
| 1 | 1.90 ± .06* | 2.26 ± .11 | 1.61 ± .06 | 1.85 ± .04 | 1.90 ± .04 | 1.61 ± .08 | 1.87 ± .04 | 1.89 ± .06 | 1.97 ± .04 | 1.88 ± .04 | 1.87 ± .02 |
| 2 | 1.80 ± .06 | 2.30 ± .11 | 1.67 ± .05 | 2.04 ± .06 | 1.68 ± .07 | 1.57 ± .07 | 1.83 ± .05 | 1.69 ± .04 | 2.04 ± .06 | 1.75 ± .05 | 1.83 ± .03 |
| 3 | 1.82 ± .04 | 2.37 ± .09 | 1.87 ± .09 | 1.84 ± .05 | 1.69 ± .08 | 1.64 ± .06 | 1.78 ± .06 | 1.76 ± .06 | 1.92 ± .08 | 1.62 ± .07 | 1.83 ± .03 |
| 4 | 1.47 ± .06 | 1.86 ± .11 | 1.36 ± .06 | 1.60 ± .05 | 1.44 ± .03 | 1.44 ± .05 | 1.62 ± .06 | 1.53 ± .07 | 1.67 ± .03 | 1.43 ± .06 | 1.53 ± .02 |
| 5a | 1.65 ± .14 | 2.09 ± .11 | 1.84 ± .06 | 2.00 ± .08 | 1.71 ± .12 | 2.16 ± .19 | 1.71 ± .08 | 1.58 ± .11 | 1.90 ± .10 | 2.06 ± .19 | 1.87 ± .04 |
| 5b | 2.75 ± .19 | 3.76 ± .33 | 3.08 ± .25 | 3.67 ± .29 | 3.47 ± .23 | 3.07 ± .21 | 2.81 ± .25 | 3.33 ± .38 | 3.18 ± .27 | 3.90 ± .39 | 3.30 ± .09 |
| 6 | 5.90 ± .27 | 7.62 ± .27 | 6.08 ± .27 | 6.29 ± .30 | 6.00 ± .13 | 5.16 ± .21 | 5.19 ± .14 | 6.56 ± .21 | 5.98 ± .27 | 5.88 ± .18 | 6.06 ± .09 |
| 7 | 5.93 ± .16 | 6.45 ± .36 | 5.70 ± .28 | 5.63 ± .31 | 5.13 ± .19 | 5.50 ± .36 | 5.23 ± .16 | 5.66 ± .10 | 5.27 ± .30 | 4.62 ± .24 | 5.51 ± .09 |
| 8 | 4.78 ± .19 | 5.61 ± .07 | 4.90 ± .10 | 4.74 ± .14 | 4.41 ± .09 | 4.57 ± .21 | 4.83 ± .18 | 5.27 ± .10 | 4.67 ± .15 | 4.58 ± .16 | 4.85 ± .05 |
| 9 | 4.87 ± .11 | 6.28 ± .09 | 4.70 ± .19 | 5.44 ± .13 | 4.74 ± .11 | 4.89 ± .17 | 5.32 ± .15 | 5.80 ± .23 | 5.11 ± .13 | 4.66 ± .13 | 5.18 ± .06 |
| 10 | 5.36 ± .22 | 6.61 ± .13 | 5.18 ± .18 | 5.65 ± .16 | 5.60 ± .13 | 5.37 ± .16 | 6.01 ± .20 | 5.57 ± .08 | 5.92 ± .23 | 5.36 ± .13 | 5.66 ± .06 |
| 11 | 5.13 ± .17 | 6.07 ± .37 | 4.97 ± .16 | 5.23 ± .38 | 5.24 ± .12 | 5.72 ± .24 | 5.95 ± .17 | 5.28 ± .36 | 5.96 ± .21 | 5.59 ± .10 | 5.52 ± .08 |
| 12 | 3.48 ± .15 | 4.20 ± .18 | 3.36 ± .12 | 3.66 ± .23 | 4.22 ± .36 | 4.17 ± .39 | 3.99 ± .26 | 3.93 ± .28 | 4.05 ± .12 | 4.39 ± .18 | 3.95 ± .08 |
| 13 | 2.55 ± .09 | 3.11 ± .14 | 2.45 ± .10 | 2.81 ± .15 | 2.59 ± .10 | 2.50 ± .16 | 2.76 ± .11 | 2.93 ± .08 | 3.02 ± .12 | 2.82 ± .16 | 2.76 ± .04 |
| All periods | 3.52 ± .12 | 4.35 ± .14 | 3.46 ± .12 | 3.67 ± .14 | 3.56 ± .12 | 3.44 ± .13 | 3.67 ± .12 | 3.80 ± .14 | 3.73 ± .13 | 3.62 ± .11 | 3.68 ± .04 |

* Standard error.

TABLE 5

*Seasonal and regional mean vitamin A and carotene contents and variability thereof in butter produced in Minnesota in 1943*

| All regions | | | | | All seasonal periods | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Seasonal period | Vitamin A | | Carotene | | Region | Vitamin A | | Carotene | |
| | Mean | S.D.* | Mean | S.D.* | | Mean | S.D.* | Mean | S.D.* |
| 1943 | μg./gm. | μg./gm. | μg./gm. | μg./gm. | | μg./gm. | μg./gm. | μg./gm. | μg./gm. |
| 1 | 4.68 | 0.57 | 1.87 | 0.31 | 1 | 6.31 | 1.36 | 3.52 | 1.76 |
| 2 | 4.65 | 0.54 | 1.83 | 0.33 | 2 | 5.67 | 1.00 | 4.35 | 2.11 |
| 3 | 4.33 | 0.52 | 1.83 | 0.34 | 3 | 6.22 | 1.39 | 3.46 | 1.78 |
| 4 | 4.86 | 0.63 | 1.53 | 0.28 | 4 | 5.99 | 1.31 | 3.67 | 1.81 |
| 5a | 5.51 | 0.94 | 1.87 | 0.45 | 5 | 6.17 | 1.75 | 3.56 | 1.72 |
| 5b | 6.83 | 1.05 | 3.30 | 1.13 | 6 | 6.49 | 1.80 | 3.44 | 1.80 |
| 6 | 7.44 | 0.82 | 6.06 | 1.10 | 7 | 6.55 | 1.94 | 3.67 | 1.78 |
| 7 | 6.88 | 1.13 | 5.51 | 1.03 | 8 | 5.83 | 1.54 | 3.80 | 1.93 |
| 8 | 7.26 | 0.92 | 4.85 | 0.65 | 9 | 6.24 | 1.67 | 3.73 | 1.74 |
| 9 | 8.06 | 1.08 | 5.18 | 0.73 | 10 | 6.58 | 1.49 | 3.62 | 1.70 |
| 10 | 8.03 | 0.90 | 5.66 | 0.75 | All regions | 6.21 | 1.57 | 3.68 | 1.83 |
| 11 | 7.80 | 1.09 | 5.52 | 0.94 | | | | | |
| 12 | 5.99 | 0.96 | 3.95 | 0.94 | | | | | |
| 13 | 5.25 | 0.68 | 2.76 | 0.50 | | | | | |
| All periods | 6.21 | 1.57 | 3.68 | 1.83 | | | | | |

* Standard deviation of single determination.

lation of region 2 than of that of the rest of the state. Indeed, in some areas of that region, Guernseys are more numerous than Holsteins. Elsewhere in the state, Holsteins predominate.

Comparison of the weighted means in table 6 with the unweighted means for all regions in table 5 indicates that the differences among regional productions of butter and among vitamin A and carotene contents thereof are so small that weighting is practically without effect.

*Seasonal fluctuations.* The data recorded in tables 4B, 4C, 5, and 6, and represented graphically in figures 2 and 3 give a general picture of seasonal fluctuations in vitamin A and carotene contents not unlike those reported by

TABLE 6

*Comparison of vitamin A and carotene contents of butters from region 2 and from the remainder of the state*

| Seasonal period | Vitamin A | | | Carotene | | |
|---|---|---|---|---|---|---|
| | Mean for region 2 | Wted. mean for | | Mean for region 2 | Wted. mean for | |
| | | Remainder of state | Entire state | | Remainder of state | Entire state |
| 1943 | μg./gm. | μg./gm. | μg./gm. | μg./gm. | μg./gm. | μg./gm. |
| 1 | 4.79 | 4.65 | 4.66 | 2.26 | 1.86 | 1.89 |
| 2 | 4.59 | 4.59 | 4.59 | 2.30 | 1.78 | 1.82 |
| 3 | 4.66 | 4.29 | 4.32 | 2.37 | 1.77 | 1.82 |
| 4 | 5.23 | 4.81 | 4.84 | 1.86 | 1.50 | 1.53 |
| 5a | 5.53 | 5.52 | 5.52 | 2.09 | 1.86 | 1.88 |
| 5b | 6.53 | 6.88 | 6.85 | 3.76 | 3.29 | 3.33 |
| 6 | 6.78 | 7.48 | 7.42 | 7.62 | 5.92 | 6.09 |
| 7 | 5.61 | 6.97 | 6.82 | 6.45 | 5.42 | 5.53 |
| 8 | 5.74 | 7.34 | 7.14 | 5.61 | 4.73 | 4.82 |
| 9 | 6.45 | 8.20 | 7.98 | 6.28 | 5.03 | 5.18 |
| 10 | 6.82 | 8.12 | 7.96 | 6.61 | 5.53 | 5.66 |
| 11 | 6.52 | 7.73 | 7.59 | 6.07 | 5.44 | 5.51 |
| 12 | 5.04 | 6.11 | 6.01 | 4.20 | 3.91 | 3.94 |
| 13 | 5.14 | 5.29 | 5.28 | 3.11 | 2.71 | 2.74 |
| All periods* | 5.73 | 6.18 | 6.14 | 4.71 | 3.58 | 3.71 |

* Weighted by seasonal production.

other workers for butters produced under similar feeding conditions.. The differences between means for the various seasonal periods are highly significant. The increases found in period 5 are, of course, due to the beginning of pasturing which occurred, except for a few herds, in approximately the same period (May 1–15) throughout the state. It seems reasonable to suppose that the peak attained in period 6, the decline in periods 7 and 8, and the rise to a second peak in periods 9 and 10, reflect fluctuations in pasture conditions although we have no objective data on the latter.

One interesting, but puzzling, fact is that in contrast to results reported by Dornbush, Peterson and Olson (13), changes in carotene content appear from our results to lag behind those in vitamin A content.

Examination of the standard deviations reported in table 5 reveals that

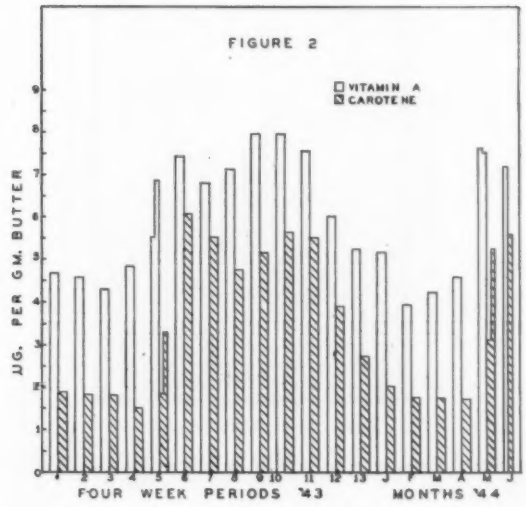



FIG. 2. Seasonal fluctuations in weighted mean vitamin A and carotene contents of Minnesota butters in 1943 and the first six months of 1944.

the winter butters were more uniform in vitamin A and carotene content than the summer butters. A similar situation has been reported for Wisconsin market milks (13).

The means weighted by seasonal butter production for the state for the entire year were 6.14 μg. of vitamin A and 3.71 μg. of carotene respectively (table 6).

Results obtained on the 126 samples analyzed during the first six months of 1944, given in table 7, have been appended to those for 1943 in figure 2

TABLE 7

*Vitamin A and carotene contents of Minnesota butters, January 1–June 15, 1944*

| Period included | No. of samples | Vitamin A | | Carotene | | Total potency |
|---|---|---|---|---|---|---|
| 1944 | | μg./gm. | I.U./lb. | μg./gm. | I.U./lb. | I.U./lb. |
| Jan. 1–31 | 20 | 5.19 ± .08* | 9,425 | 2.01 ± .07* | 1,521 | 10,946 |
| Feb. 1–29 | 20 | 3.97 ± .12 | 7,210 | 1.76 ± .06 | 1,332 | 8,542 |
| Mar. 1–31 | 19 | 4.22 ± .08 | 7,664 | 1.74 ± .04 | 1,317 | 8,981 |
| Apr. 1–30 | 20 | 4.60 ± .06 | 8,354 | 1.70 ± .05 | 1,286 | 9,640 |
| May 1–15 | 10 | 7.68 ± .26 | 13,947 | 3.11 ± .23 | 2,354 | 16,301 |
| May 16–22 | 9 | 7.61 ± .18 | 13,820 | 4.45 ± .25 | 3,368 | 17,198 |
| May 23–31 | 10 | 7.45 ± .17 | 13,529 | 6.00 ± .24 | 4,541 | 18,070 |
| June 1– 8 | 10 | 7.30 ± .15 | 13,257 | 5.75 ± .29 | 4,352 | 17,609 |
| June 9–15 | 8 | 7.08 ± .19 | 12,857 | 5.41 ± .30 | 4,094 | 16,951 |

* Standard error.

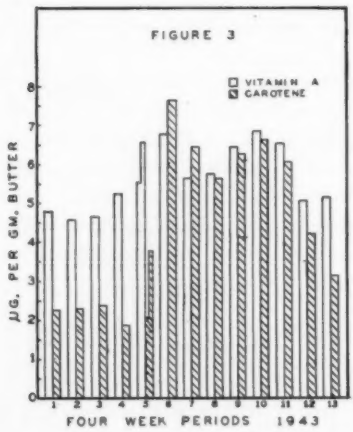



FIG. 3. Seasonal fluctuations in vitamin A and carotene contents of butters from region 2 in 1943.

for comparative purposes. Contrary to expectation based on knowledge of roughage quality in the two years, the vitamin A and carotene contents were not appreciably different during the 1944 winter season than during that of 1943.

*Total vitamin A potency.* In order to express potencies in single figures,

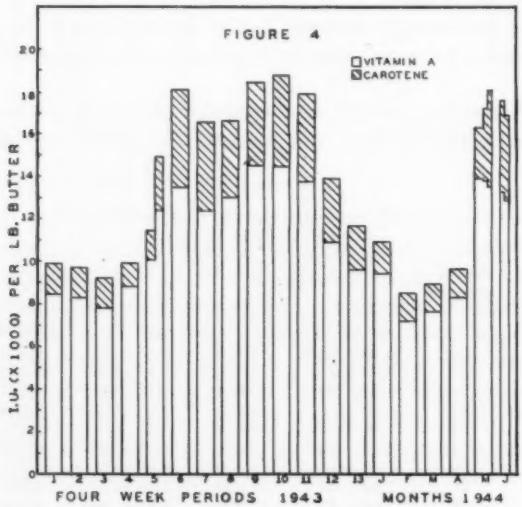



FIG. 4. Seasonal fluctuations in total vitamin A potency of Minnesota butters in 1943 and the first six months of 1944.

TABLE 8

*Total vitamin A potency of Minnesota butters in 1943*

| Seasonal period | Mean vitamin A potency | | | Per cent of total as | | Distribution of potencies of samples among ranges of International Units per pound | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Vitamin A | Carotene | Total | Vitamin A | Carotene | Up to 9,000 | 9,001 to 12,000 | 12,001 to 15,000 | 15,001 to 18,000 | 18,001 to 21,000 | 21,001 and over |
| 1943 | *I.U./lb.* | *I.U./lb.* | *I.U./lb.* | % | % | % | % | % | % | % | % |
| 1 | 8,462 | 1,430 | 9,892 | 85.5 | 14.5 | 21.0 | 76.5 | 2.5 | ..... | ..... | ..... |
| 2 | 8,335 | 1,377 | 9,712 | 85.2 | 14.2 | 24.0 | 76.0 | ..... | ..... | ..... | ..... |
| 3 | 7,845 | 1.377 | 9,222 | 85.1 | 14.9 | 44.2 | 54.5 | 1.3 | ..... | ..... | ..... |
| 4 | 8,789 | 1,158 | 9,947 | 88.4 | 11.6 | 26.0 | 70.1 | 3.9 | ..... | ..... | ..... |
| 5a | 10,024 | 1,422 | 11,446 | 87.8 | 12.4 | 7.0 | 56.1 | 31.5 | 5.3 | ..... | ..... |
| 5b | 12,440 | 2,520 | 14,960 | 83.2 | 16.8 | 1.4 | 9.7 | 47.2 | 33.3 | 8.3 | ..... |
| 6 | 13,475 | 4,608 | 18,083 | 74.5 | 25.5 | ..... | ..... | 1.3 | 45.9 | 50.0 | 2.8 |
| 7 | 12,385 | 4,184 | 16,569 | 74.7 | 25.3 | ..... | ..... | 23.9 | 54.9 | 18.3 | 2.8 |
| 8 | 12,966 | 3,647 | 16,613 | 78.0 | 22.0 | ..... | ..... | 15.1 | 57.5 | 27.4 | ..... |
| 9 | 14,492 | 3,920 | 18,412 | 78.7 | 21.3 | ..... | ..... | 2.9 | 42.6 | 48.5 | 5.9 |
| 10 | 14,455 | 4,283 | 18,738 | 77.1 | 22.9 | ..... | ..... | 4.0 | 23.0 | 63.5 | 9.5 |
| 11 | 13,783 | 4,169 | 17,952 | 76.8 | 23.2 | ..... | 1.4 | 6.8 | 35.6 | 45.2 | 11.0 |
| 12 | 10,914 | 2,981 | 13,895 | 78.5 | 21.5 | ..... | 18.1 | 59.7 | 16.7 | 5.6 | ..... |
| 13 | 9,588 | 2,073 | 11,661 | 82.2 | 17.8 | 1.4 | 53.5 | 43.7 | 1.4 | ..... | ..... |
| All periods* | 11,150 | 2,808 | 13,958 | 79.9 | 20.1 | 10.4 | 30.7 | 14.4 | 23.6 | 18.9 | 2.0 |

* Weighted by seasonal production.

the weighted means for the entire state have been converted to International Units per pound of butter. In making this conversion, the potencies of carotene and vitamin A were considered equal to 1.667 and 4.000 I.U./μg. respectively. The latter value, based on results obtained by the Technical Committee in rat feeding experiments with six butterfat samples,[7] is admittedly tentative, but it is approximately the average of the values of 3.3 I.U./μg. reported by Mead *et al.* (27) and that of 4.3 I.U./μg. found by Baxter and Robeson (4, 5). Furthermore, use of this value makes our results comparable to those of Berl and Peterson (6).

The contributions of carotene and of the vitamin itself to the total vitamin A potency are given in table 8 and illustrated in figure 4. Similar data for the 1944 samples are shown in table 7 and appended to figure 4. The seasonal fluctuations are apparent. Not only does the total potency fluctuate, but also the distribution of potency between vitamin A and carotene varies widely with season. Our values for the potencies of butters produced in periods 1, 3, 7 and 10 correspond rather closely to those of Wisconsin butters produced in January, March, July and September respectively (6).

Table 8 also presents the percentage frequency distribution of the samples among various ranges of potency. At least 75 per cent of the samples in any one period lie within a range of 6,000 I.U./lb. except in period 7. The percentage distribution of the potency of all 1943 butter, weighted for seasonal production, has been computed and is presented at the foot of table 8.

*Total production of vitamin A and carotene.* It is of some interest to compute both the total annual production of vitamin A and carotene in the

TABLE 9

*Seasonal distribution of Minnesota's production of butter and of vitamin A and carotene therein during 1943*

| Seasonal period | Butter production | | Vitamin A | | | Carotene | | |
|---|---|---|---|---|---|---|---|---|
| | | | Content | Production | | Content | Production | |
| | *lbs.* × *1,000* | % | *μg./gm.* | *lbs.* | % | *μg./gm.* | *lbs.* | % |
| 1 | 23,084 | 7.84 | 4.66 | 107.6 | 5.95 | 1.89 | 43.6 | 3.99 |
| 2 | 24,859 | 8.45 | 4.59 | 114.1 | 6.31 | 1.82 | 45.2 | 4.14 |
| 3 | 26,191 | 8.90 | 4.32 | 113.1 | 6.26 | 1.82 | 47.7 | 4.37 |
| 4 | 26,276 | 8.93 | 4.84 | 127.2 | 7.04 | 1.53 | 40.2 | 3.68 |
| 5a | 13.829 | 4.70 | 5.52 | 76.3 | 4.22 | 1.88 | 26.0 | 2.38 |
| 5b | 14.528 | 4.94 | 6.85 | 99.5 | 5.51 | 3.33 | 48.4 | 4.43 |
| 6 | 31,311 | 10.64 | 7.42 | 232.3 | 12.86 | 6.09 | 190.7 | 17.47 |
| 7 | 29,552 | 10.04 | 6.82 | 201.5 | 11.15 | 5.53 | 163.4 | 14.97 |
| 8 | 24,261 | 8.24 | 7.14 | 173.2 | 9.58 | 4.82 | 116.9 | 10.71 |
| 9 | 19,532 | 6.64 | 7.98 | 155.9 | 8.63 | 5.18 | 101.2 | 9.27 |
| 10 | 16,134 | 5.48 | 7.96 | 128.4 | 7.11 | 5.66 | 91.3 | 8.36 |
| 11 | 13,622 | 4.63 | 7.59 | 103.4 | 5.72 | 5.51 | 75.0 | 6.87 |
| 12 | 14,075 | 4.78 | 6.01 | 84.6 | 4.68 | 3.94 | 55.5 | 5.08 |
| 13 | 17,012 | 5.78 | 5.28 | 89.8 | 4.97 | 2.74 | 46.6 | 4.27 |
| Total | 294,270 | 100.00 | ..... | 1806.9 | 100.00 | ..... | 1091.7 | 100.00 |

[7] Unpublished data.

TABLE 10

*Effect of storage on vitamin A and carotene contents of butter*

| Month produced | No. of tubs | Mean vitamin A content after | | | Mean carotene content after | | |
|---|---|---|---|---|---|---|---|
| | | 0 months | 3 months | 6 months | 0 months | 3 months | 6 months |
| 1943 | | μg./gm. | μg./gm. | μg./gm. | μg./gm. | μg./gm. | μg./gm. |
| January | 9 | 4.73 ± .09* | 4.65 ± .12 | 4.46 ± .08 | 1.98 ± .10* | 1.90 ± .09 | 1.88 ± .07 |
| February | 9 | 3.98 ± .18 | 4.43 ± .10 | 4.46 ± .13 | 1.84 ± .12 | 1.74 ± .14 | 2.03 ± .14 |
| March | 9 | 4.33 ± .12 | 4.35 ± .05 | 4.63 ± .08 | 1.73 ± .09 | 1.68 ± .10 | 1.66 ± .10 |
| April | 9 | 4.84 ± .16 | 4.51 ± .12 | 4.62 ± .12 | 1.75 ± .10 | 1.75 ± .09 | 1.81 ± .08 |
| May | 7 | 7.29 ± .18 | 7.41 ± .12 | 7.85 ± .20 | 5.80 ± .16 | 5.82 ± .19 | 5.55 ± .16 |
| June | 9 | 6.26 ± .17 | 7.41 ± .19 | 7.52 ± .26 | 5.39 ± .16 | 5.53 ± .18 | 5.19 ± .14 |
| All months | 52 | 5.16 ± .12 | 5.39 ± .14 | 5.50 ± .16 | 2.98 ± .18 | 2.97 ± .18 | 2.93 ± .16 |

* Standard error.

state's butter and the seasonal distribution thereof. This estimate has been compiled in table 9. The fact that the high point in butter production coincides with one of the peaks in vitamin A and carotene content, results in a decided concentration of the production of the vitamin and its provitamin in periods 6 and 7.

The total annual production amounted to nearly a ton of vitamin A and over a half ton of carotene.

*Effect of storage.* One ten-pound tub of butter was received from each region[8] each month during the first six months of 1943. After withdrawal of a sample for immediate analysis, the tubs were placed in storage at – 10° F., and other samples were withdrawn and analyzed after storage periods of three and six months. The results of this study, presented in table 10, show that the carotene content of these butters was little affected by storage. Statistical analysis revealed that the differences attributable to the effects of storage were insignificant. In the case of the vitamin A content, the very disturbing anomalous *increases* noted in the February, May, and June samples after storage must be due to an uncontrolled error in the experiment. For the data as a whole, however, the differences in vitamin A content due to storage are statistically insignificant. Certainly there is no evidence of deterioration in potency during storage.

## DISCUSSION

It appears that Minnesota, with the exception of two regions together producing about 20 per cent of its butter, may be considered a homogeneous area insofar as the carotene content of its butter is concerned. The two exceptional regions, while both producing butter of carotene content higher than the state average, differ significantly from one another in this regard. While there is somewhat greater variability among regions in vitamin A content than in carotene content, the regional differences are probably not of practical significance.

The seasonal fluctuations in potency of butter produced in the state are not unlike those reported from other areas in similar latitudes in other parts of the world. In particular, they closely resemble fluctuations observed in butters and market milks in the neighboring state of Wisconsin.

We are unable to explain why our results, indicating that changes in carotene content lag behind those in vitamin A, are so distinctly different from those reported from Wisconsin. Indeed, it seems fruitless to hazard a guess as to the underlying reason in view of the fact that the samples, collected at random, did not necessarily represent the same herds in successive periods.

Only about 10 per cent of the butter produced in the state in 1943 fell below the commonly accepted minimum of 9,000 I.U. per pound. However,

[8] Except region 4.

in view of demonstrations (21, 31) that the potency can be maintained at summer levels in winter merely by feeding high quality roughage, it is not encouraging to note that as much as 44 per cent of the butter produced in one four-week period fell below the 9,000 I.U. level.

## SUMMARY AND CONCLUSIONS

A survey of the vitamin A and carotene contents of creamery butter produced in Minnesota in 1943 and 1944 led to the following conclusions:

1. In 1,019 samples analyzed in 1943 the unweighted mean vitamin A and carotene contents were respectively $6.21 \pm 0.03$ and $3.68 \pm 0.04$ μg. per gm. The means weighted by regional and seasonal butter production were respectively 6.14 and 3.71 μg. per gm.; these values correspond to a total potency of 13,958 I.U. per pound if vitamin A itself is considered to have a potency of 4.00 I.U. per μg.

2. With a few exceptions, and for all practical purposes, the butters of the state were rather uniform in vitamin A potency in any given season of the year.

3. Marked seasonal fluctuations in potency, similar to those reported elsewhere, and undoubtedly attributable to variations in feeding conditions were found to occur. From January 1 to April 22, 1943, the samples averaged 9,000–10,000 I.U. per pound. With the beginning of pasturing, a rapid rise occurred until the summer level of 16,500–18,500 I.U. per pound was attained in the period May 21 to June 17. This level persisted until early October whereupon the potency gradually declined nearly to the winter level during the remainder of the year. The picture for the first half of the year was corroborated by analyses of samples secured from January 1 to June 15, 1944.

4. The distribution of total potency between vitamin A and carotene varied with season. In winter butter carotene furnished only 11 to 15 per cent of the potency while in summer butter it furnished 21 to 25 per cent of it.

In general changes in carotene content lagged behind those in vitamin A content.

5. It was calculated that about 90 per cent of the butter produced in 1943 had vitamin A potencies of more than 9,000 I.U. per pound; about 45 per cent of it had potencies greater than 15,000 I.U. per pound.

6. It was estimated that 1,800 pounds of vitamin A and 1,100 pounds of carotene were produced in Minnesota butter in 1943.

7. No evidence was found for loss of vitamin A potency by butter during storage.

## ACKNOWLEDGMENTS

It is a pleasure to acknowledge the invaluable assistance given by Prof. W. B. Combs, Division of Dairy Husbandry, University Farm, in organizing the program for the collection of butter samples for this survey.

We wish also to express our appreciation to Dr. W. F. Geddes, Division of Agricultural Biochemistry, University Farm, for his advice relative to summarization of the data and preparation of the manuscript.

The cooperation of the several creameries in submitting samples and of the Minnesota Dairy Industry Committee in financing the project has been appreciated.

## REFERENCES

(1) ARCHIBALD, J. G., AND PARSONS, C. H. The Effect of Feeding a Vitamin A Supplement to Dairy Cattle. Mass. Agr. Expt. Sta., Bul. 357. 1939.

(2) BAUMANN, C. A., AND STEENBOCK, H. Fat-Soluble Vitamins. XXXVI. The Carotene and Vitamin A Content of Butter. Jour. Biol. Chem., **101**: 547–560. 1933.

(3) BAUMANN, C. A., STEENBOCK, H., BEESON, W. M., AND RUPEL, I. W. Fat-Soluble Vitamins. XXXIX. The Influence of Breed and Diet of Cows on the Carotene and Vitamin A Content of Butter. Jour. Biol. Chem., **105**: 167–176. 1934.

(4) BAXTER, J. G., AND ROBESON, C. D. Crystalline Aliphatic Esters of Vitamin A. Amer. Chem. Soc. Jour., **64**: 2407–2410. 1942.

(5) BAXTER, J. G., AND ROBESON, C. D. Crystalline Vitamin A. Amer. Chem. Soc. Jour., **64**: 2411–2416. 1942.

(6) BERL, S., AND PETERSON, W. H. Determination and Content of Carotene and Vitamin A in Wisconsin Butter. Jour. Nutr., **26**: 527–537. 1943.

(7) BOOTH, R. G., KON, S. K., AND GILLAM, A. E. The Relative Biological Efficiencies of the Vitamin A and Carotene of Butter. Biochem. Jour., **28**: 2169–2174. 1934.

(8) BOOTH, R. G., KON, S. K., DANN, W. J., AND MOORE, T. A Study of Seasonal Variations in Butter Fat. I. Seasonal Variations in Carotene, Vitamin A and the Antimony Trichloride Reaction. Biochem. Jour. **27**: 1189–1196. 1933.

(9) DAVIS, H. P., AND HATHAWAY, I. L. The Vitamin A Content of the Milk of Holstein, Ayrshire, Jersey, and Guernsey Cows. Neb. Agr. Expt. Sta., Res. Bul. 54. 1931.

(10) DEUEL, H. J., JR., HALLMAN, L. F., JOHNSTON, C., AND MATTSON, F. The Effect of a High Vitamin A Intake on the Blood and Milk Carotene of Holstein and Guernsey Cows. Jour. Nutr., **23**: 567–579. 1942.

(11) DEUEL, H. J. JR., HALLIDAY, N., HALLMAN, L. F., JOHNSTON, C., AND MILLER, A. J. The Effect of Vitamin A Intake on Vitamin A Content of Butter Fat. Jour. Biol. Chem., **139**: 479–480. 1941.

(12) DEUEL, H. J. JR., HALLIDAY, N., HALLMAN, L. F., JOHNSTON, C., AND MILLER, A. J. The Production of High Vitamin A Milk by Diet. Jour. Nutr., **22**: 303–313. 1941.

(13) DORNBUSH, A. C., PETERSON, W. H., AND OLSON, F. R. The Carotene and Vitamin A Content of Market Milks. Amer. Med. Assoc. Jour., **114**: 1748–1751. 1940.

(14) FOUNTAINE, F. C., AND BOLIN, D. W. The Milk and Butterfat Production Responses to Shark Liver Oil in the Ration. JOUR. DAIRY SCI., **27**: 155–158. 1944.

(15) FRAPS, G. S., COPELAND, O. C., AND TREICHLER, R. The Vitamin A Requirements of Dairy Cows. Tex. Agr. Expt. Sta., Bul. 495. 1934.

(16) FRAPS, G. S., AND TREICHLER, R. Quantitative Variations in Vitamin A Content of Butter Fat. Indus. and Engin. Chem., **24**: 1079–1081. 1932.

(17) GILLAM, A. E., HEILBRON, I. M., MORTON, R. A., BISHOP, G., AND DRUMMOND, J. C. Variations in the Quality of Butter, Particularly in Relation to the Vitamin A, Carotene and Xanthophyll Content as Influenced by Feeding Artificially Dried Grass to Stall-Fed Cattle. Biochem. Jour., **27**: 878–888. 1933.

(18) GOLDING, J., SOAMES, K. M., AND ZILVA, S. S. The Influence of the Cow's Diet on the Fat Soluble Vitamins of Winter Milk. Biochem. Jour., 20: 1306–1319. 1926.

(19) HATHAWAY, I. L., AND DAVIS, H. P. The Vitamin A Content of Skimmilk, Standardized Milk and Cream from Different Breeds of Cows. Neb. Agr. Expt. Sta., Res. Bul. 69. 1933.

(20) HAUGE, S. M., WESTFALL, R. J., WILBUR, J. W., AND HILTON, J. H. The Vitamin A Requirements of Dairy Cows for Production of Butterfat of High Vitamin A Value. III. Availability of Carotene in Dehydrated Alfalfa Hay as Compared with Carotene in Oil. JOUR. DAIRY SCI., 27: 63–66. 1944.

(21) HILTON, J. H., HAUGE, S. M., AND WILBUR, J. W. Maintaining the Vitamin A Value of Butter Through Winter Feeding Conditions. JOUR. DAIRY SCI., 16: 355–361. 1933.

(22) HILTON, J. H., WILBUR, J. W., AND HAUGE, S. M. The Vitamin A Requirements of Dairy Cows for Production of Butterfat of High Vitamin A Value. II. Vitamin A *Per Se*. JOUR. DAIRY SCI., 27: 57–62. 1944.

(23) KEMMERER, A. R., AND FRAPS, G. S. The Vitamin A Content of Commercial Butters Sold in Texas. Tex. Agr. Expt. Sta., Bul. 629. 1943.

(24) KOEHN, C. J. Vitamin A Activity of Milk as Related to Pasture and Feeding Practices in Alabama. JOUR. DAIRY SCI., 26: 673–681. 1943.

(25) KOEHN, C. J., AND SHERMAN, W. C. The Determination of Vitamin A and Carotene with the Photoelectric Colorimeter. Jour. Biol. Chem., 132: 527–538. 1940.

(26) LOY, W. C., HILTON, J. H., WILBUR, J. W., AND HAUGE, S. M. The Rate of Change in the Vitamin A Content of Milk. JOUR. DAIRY SCI., 20: 31–36. 1937.

(27) MEAD, T. H., UNDERHILL, S. W. F., AND COWARD, K. H. Crystalline Esters of Vitamin A. Biochem. Jour., 33: 589–600. 1939.

(28) MITCHELL, J. H., AND WISE, G. H. The Comparative Effects of Continual and Rotational Systems of Grazing on the Carotene Content of Permanent Pasture Herbage and of the Milk Produced Therefrom. JOUR DAIRY SCI., 27: 189–196. 1944.

(29) MOORE, T. Vitamin A and Carotene. IX. Notes on the Conversion of Carotene to Vitamin A in the Cow. Biochem. Jour., 26: 1–9. 1932.

(30) MORGAN, R. S., AND PRITCHARD, H. The Average Vitamin A and Vitamin D Potency of Butter. Analyst, 62: 354–362. 1937.

(31) MUNIN, F. Natural Methods for Increasing the Vitamin A Content of Butterfat. Fette u. Seifen, 50: 288–290. 1943. (Original not seen.)

(32) PETERSON, W. H., BIRD, H. R., AND BEESON, W. M. Chemical Changes in the Making of A.I.V. Alfalfa Silage and Nutritive Qualities of Milk Produced Therefrom. JOUR. DAIRY SCI., 20: 611–623. 1937.

(33) RUSSELL, W. C., TAYLOR, M. W., CHICHESTER, D. C., AND WILSON, L. T. Relation Between the Vitamin A Content of the Dairy Ration and That of Milk. N. J. Agr. Expt. Sta., Bul. 592. 1935.

(34) RUSOFF, L. L., SKIPPER, H. E., AND DIX ARNOLD, P. T. Shark Liver Oil and the Vitamin A Potency of Milk. JOUR. DAIRY SCI., 25: 807–813. 1942.

(35) SUTTON, T. S., AND KRAUSS, W. E. Comparative Study of the Vitamin A Content of Butterfat from Four Breeds of Dairy Cattle. Ohio Agr. Expt. Sta., Bul. 548: 59. 1935.

(36) TAINSH, P. W., AND WILKINSON, M. H. The Vitamins Associated with Natural Oils and Fats. Compt. Rend. 5th Cong. Internatl. Tech. Chem. Indus. Agr., 3: 263–275. 1937.

(37) WATSON, S. J., BISHOP, G., DRUMMOND, J. C., GILLAM, A. E., AND HEILBRON, I. M. The Relation of the Color and Vitamin A Content of Butter to the Nature of the Ration Fed. Biochem. Jour., **28**: 1076–1085. 1934.

(38) WILBUR, J. W., HILTON, J. H., AND HAUGE, S. M. The Vitamin A Activity of Butter Produced by Guernsey and Ayrshire Cows. JOUR. DAIRY SCI., **16**: 153–156. 1933.

(39) WILKINSON, H. The Vitamin A and Vitamin D Contents of Butter. II. Seasonal Variation. Analyst, **64**: 17–23. 1939.

(40) ZSCHEILE, F. P., NASH, H. A., HENRY, R. L., AND GREEN, L. F. Determination of Vitamin A and Carotenoids in Butterfat. Comparison of Direct Spectrophotometry with Filter Photometry and Use of the Antimony Trichloride Reaction. Indus. and Engin. Chem., Analyt. Ed., **16**: 83–85. 1944.

# THE RELATIONSHIP BETWEEN A LOW CAROTENE INTAKE AND URINARY EXCRETION OF ASCORBIC ACID IN DAIRY CATTLE*

L. A. MOORE AND J. W. COTTER
*Dairy Department, University of Maryland*

The possibility of a relationship between vitamin A intake and ascorbic acid metabolism and reproduction in animals has recently appeared in the scientific literature. Phillips and co-workers (19) reported lower blood plasma and urine ascorbic acid values in a calf on a vitamin A deficient ration than in a check animal which received 100 micrograms of carotene per kilo of body weight. The storage of riboflavin in the liver of the vitamin A deficient calf was also lower than in the check animal. Sure (23) demonstrated a decreased ascorbic acid content of the blood plasma and tissues of the vitamin A, $B_1$ and riboflavin deficient rat. Sutton (24) fed rats various amounts of vitamin A and noted that urinary excretion of ascorbic acid was less when the rats received 10 and 20 International Units of vitamin A per kilo of body weight, than when 30 units were fed. Phillips (18) reported that the feeding of a high potency shark liver oil to certain dairy herds in a breeding cooperative caused the animals to maintain more nearly normal blood-plasma ascorbic acid values. Boyer and co-workers (4) found lowered blood plasma and cerebrospinal fluid ascorbic acid values in calves on a vitamin A free ration. The urinary excretion of ascorbic acid in rats on a vitamin A deficient diet was considerably lower than in control animals receiving vitamin A.

On the other hand, Hart, Goss, and Guilbert (6) working with horses demonstrated that a low vitamin A intake carried to a fatal termination did not seriously affect the blood levels of ascorbic acid. Rubin and Bird (21) found the ascorbic acid content of the livers and duodena of vitamin A deficient hens to be as great as that of the same organs of hens fed ample vitamin A. These data, the authors indicate, show that vitamin A deficiency in the mature chicken does not interfere with ascorbic acid synthesis. Jensen and co-workers (7) were not able to increase the plasma ascorbic acid values of dairy cows by feeding vitamin A in the form of shark liver oil. Moore, Berry and Sykes (16) found no particular relationship between blood plasma ascorbic values and vitamin A intake in calves receiving from 44 to 75 micrograms of carotene per kilo of body weight. Kuhlman and Gallup (12) have pointed out that a satisfactory conception rate was obtained when the carotene intake was at the same level as that required

Received for publication December 16, 1944.

* Scientific Paper No. A94, Contribution No. 1953, of the Maryland Agricultural Experiment Station (Department of Dairy Husbandry).

for normal calving. A study of the data reported by Jones, Haag and Brandt (8) does not show that the feeding of cod liver oil to cows on restricted rations improved the conception rate. Jones, Haag and Daugherty (9) reported that a ration which was adequate for normal growth in bovine males was satisfactory for normal reproductive performance.

Because of the possible relationships between vitamin A intake and ascorbic acid synthesis in the tissues and because of the relationship of ascorbic acid to reproduction, it was decided to study the urinary excretion of ascorbic acid in dairy animals fed normal winter rations and on various low levels of carotene intake. The results of such a study are herein reported.

## EXPERIMENTAL

Urine collections were made on heifers receiving grain, corn silage and clover and timothy hay. Collections were also made on heifers receiving a carotene-free grain mixture, 10 lbs. wheat straw and carotene in alfalfa leaf meal equal to 42 micrograms per kilo of body weight. Urine collections were also made on males receiving normal winter rations and on various low intakes of carotene. In some instances various amounts of chlorobutanol were fed in the grain. This compound has been shown by Longnecker (13) to stimulate ascorbic acid excretion in rats and to increase the blood plasma ascorbic acid values in dairy cattle (3).

The 24-hour collection periods varied in length from two to six days. In the females the urine was generally collected by massage in order to prevent loss. In the males the urine was collected in rubber bags made from inner tubes which were strapped to the body. As soon as the urine was collected, the volume was obtained and an aliquot taken which was placed in a composite bottle. Ten per cent of 5 N $H_2SO_4$ was added to each aliquot. The composite bottles were stored in ice water until the end of each 24-hour period after which the ascorbic acid determinations were made.

The ascorbic acid determinations were first made by a modification of the method of Mindlin and Butler (14). The fading of the dye was measured by a photoelectric spectrophotometer using a wave length of 510 millimicrons. The presence of urine pigments was taken into account. In later phases of the work the method of Roe and Kuether (20) was used. The two methods checked within reason. Blood plasma ascorbic acid determinations were also made by the method of Mindlin and Butler as modified by Golden and Garfinkel (5). Ascorbic acid determinations on whole blood were made by the method of Roe and Keuther (20). Vitamin A and carotene determinations were made by a previously published procedure (15) modified as suggested by Kimble (10).

The animals used were generally of the Holstein breed but one Ayrshire, one Jersey and one Guernsey male were included. The breed of the animal is indicated by the letters H, A, J or G following the name or number of the animal.

## RESULTS AND DISCUSSION

The results obtained with the heifers fed normal winter rations are shown in table 1 in which the data have been arranged according to stage of gestation. The excretion of ascorbic acid varied considerably in this group of

TABLE 1

*Urinary excretion of ascorbic acid by dairy heifers*

| Animal No. | Weight | Stage of gestation | Ascorbic acid excreted per day | | Ascorbic acid excreted per day per kilo | | Plasma ascorbic acid | |
|---|---|---|---|---|---|---|---|---|
| | | | 1st day | 2nd day | 1st day | 2nd day | 1st day | 2nd day |
| | *lbs.* | *days* | *mg.* | *mg.* | *mg.* | *mg.* | *mg./100 ml.* | *mg./100 ml.* |
| **Normal ration** | | | | | | | | |
| 69H | 858 | 7 | 44.6 | 33.4 | 0.11 | 0.09 | 0.41 | 0.37 |
| 68H | 836 | 83 | 38.9 | 33.4 | 0.10 | 0.09 | 0.38 | 0.27 |
| 67H | 882 | 94 | 31.0 | 27.0 | 0.08 | 0.07 | 0.37 | 0.37 |
| 66H | 1067 | 98 | 58.0 | 39.7 | 0.12 | 0.08 | ..... | ..... |
| 64H | 1105 | 142 | 110.5 | 67.7 | 0.22 | 0.13 | ..... | ..... |
| 69H | 1004 | 146 | 57.3 | 72.0 | 0.13 | 0.16 | 0.39 | 0.29 |
| 82A | 916 | 159 | 52.9 | 47.4 | 0.13 | 0.11 | 0.32 | 0.39 |
| 62H | 1120 | 217 | 42.8 | 45.4 | 0.08 | 0.09 | 0.23 | 0.26 |
| 68H | 1062 | 222 | 42.3 | 48.7 | 0.09 | 0.10 | 0.36 | 0.37 |
| 60H | 1300 | 231 | 56.9 | 38.4 | 0.10 | 0.06 | 0.26 | 0.27 |
| 67H | 1234 | 234 | 52.4 | 64.7 | 0.09 | 0.12 | 0.37 | 0.37 |
| 60H | 1323 | 237 | 30.7 | 38.2 | 0.05 | 0.06 | 0.27 | ..... |
| 48G | 1062 | 250 | 64.5 | 48.1 | 0.13 | 0.10 | 0.36 | 0.32 |
| 41G | 1066 | 254 | 43.8 | 25.8 | 0.09 | 0.05 | 0.38 | 0.39 |
| 70A | 1195 | 258 | 45.5 | 47.9 | 0.08 | 0.09 | 0.46 | 0.33 |
| **Normal ration plus 5 gm. chlorobutanol per day for ten days** | | | | | | | | |
| 66H | 1067 | 120 | 315.4 | 210.0 | 0.65 | 0.43 | 0.64 | 0.42 |
| 64H | 1148 | 164 | 399.0 | 348.1 | 0.76 | 0.67 | 0.61 | 0.46 |
| **Low carotene ration plus alfalfa meal** | | | | | | | | |
| 74A | 1040 | Open | 80.3 | 79.0 | 0.17 | 0.17 | ..... | ..... |
| 54H | 1230 | 174 | 177.8 | 198.0 | 0.32 | 0.35 | ..... | ..... |
| 54H | 1275 | 196 | 310.6 | 266.8 | 0.53 | 0.46 | 0.34 | 0.25 |
| 57H | 1280 | 213 | 140.6 | 189.6 | 0.24 | 0.33 | ..... | ..... |
| 57H | 1300 | 235 | 288.5 | 281.2 | 0.49 | 0.48 | 0.29 | 0.39 |
| **Low carotene ration plus alfalfa meal plus 5 gm. chlorobutanol per day for ten days** | | | | | | | | |
| 74A | 1070 | 19 | 377.3 | 312.1 | 0.78 | 0.64 | 0.42 | 0.41 |
| 74A | 1090 | 32 | 328.5 | 333.0 | 0.58 | 0.67 | 0.38 | ..... |
| 54H | 1300 | 209 | 247.7 | 232.6 | 0.45 | 0.39 | 0.20 | ..... |
| 57H | 1320 | 248 | 274.4 | 494.0 | 0.48 | 0.82 | 0.42 | ..... |

animals since the values range from a low of 25.8 to a high of 110 milligrams per day. There does not appear to be any particular relationship between the stage of gestation and the excretion of ascorbic acid. Within the group there does not appear to be any particular relationship between the blood plasma values and the amount of ascorbic acid excreted. The excretion

values agree with those reported by Knight, Dutcher and Guerrant (11) on cows but are somewhat higher than those reported by Wallis (25).

When 5 grams of chlorobutanol per day were fed to two of the heifers 64H and 66H, the excretion of ascorbic acid was increased 4 to 8 fold. The blood plasma ascorbic acid values likewise showed a considerable increase as shown in table 2. These results correspond to those previously reported by Longnecker (13), Sutton (24) and Bortree (3).

The excretion of ascorbic acid by 74A, an open animal, fed a ration containing about 42 micrograms of carotene per kilo of body weight was similar to the normally fed animals. However, in the other two animals which had been pregnant for varying periods of time and receiving 42 micrograms of carotene per kilo of body weight, the excretion of ascorbic acid was 4 to 6 times that of the normally fed heifers. When these three

TABLE 2

*Effect of five grams of chlorobutanol on blood plasma ascorbic values of normally fed and low carotene intake pregnant heifers*

| Date | 64H | 66H | Date | 57H | 54H |
|---|---|---|---|---|---|
| | Normally fed | | | Low carotene intake | |
| | *Milligrams ascorbic acid per 100 ml. plasma* | | | | |
| 11/12/42 | 0.35 | 0.22 | 11/25/42 | 0.31 | 0.24 |
| 11/18/42 | 0.37 | 0.44 | 11/27/42 | 0.29 | 0.34 |
| 11/18/42 | Chlorobutanol added | | 12/ 2/42 | Chlorobutanol added | |
| 11/20/42 | 0.48 | 0.61 | 12/ 4/42 | 0.36 | 0.25 |
| 11/23/42 | 0.51 | 0.61 | 12/ 9/42 | 0.42 | 0.25 |
| 11/27/42 | 0.61 | 0.64 | 12/11/42 | ..... | 0.20 |
| 11/30/42 | Chlorobutanol discont. | | 12/12/42 | Chlorobutanol discont. | |
| 12/ 4/42 | 0.55 | 0.48 | 12/15/42 | 0.34 | 0.27 |
| 12/ 9/42 | 0.42 | 0.52 | 12/23/42 | 0.35 | 0.28 |
| 12/15/42 | 0.31 | 0.35 | 1/ 4/43 | 0.32 | 0.22 |

animals were fed chlorobutanol, ascorbic acid excretion was markedly increased in 74A which had been pregnant for only 2½ weeks. This reaction was similar to the normally fed animals. However, the chlorobutanol did not materially increase the excretion of ascorbic acid of the other two heifers which were further along in the gestation period. The plasma ascorbic acid values for the low carotene fed heifers were not elevated as shown in table 2 even though they excreted large quantities of ascorbic acid. In unpublished data this had previously been noted in pregnant heifers on a low carotene ration. This would indicate a lack of relationship between plasma ascorbic acid values and excretion, possibly due to altered kidney threshold in these particular animals. Just why ascorbic acid synthesis should be stimulated in pregnant heifers fed a low carotene ration is not clear. This effect is just the opposite of that reported by Boyer (4) and Sutton (24) in rats and in severely deficient male calves as noted in this paper.

The urinary excretion of ascorbic acid by dairy males was also studied. The results of three different two-day collection periods on two animals

seven to fourteen months of age receiving a normal winter ration are shown in table 3. These results indicate considerable variation and the excretion per kilo of body weight was considerably higher than for normally fed females. A later collection from these same two males for a six-day period was made, the results of which are shown in table 4. Practically the same variations were noted during this period. In addition, ascorbic determinations were made on whole blood and on plasma, and vitamin A and carotene determinations were made on the blood plasma as shown in table 4.

Data were also collected on a seven-year-old Jersey male which had become a slow breeder. The ascorbic acid excretion in this animal seemed to be somewhat depressed especially when the results were calculated on the body weight basis. The excretion varied from 0.10 to 0.21 mg. per kilo per day, values which are below any values obtained on the other normal

TABLE 3

*Urinary excretion of ascorbic acid by young males fed a normal ration*

| Animal No. | Age | Weight | Ascorbic acid excreted per day | | Ascorbic acid excreted per day per kilo of body weight | |
|---|---|---|---|---|---|---|
| | | | 1st day | 2nd day | 1st day | 2nd day |
| | *mo.* | *lbs.* | *mg.* | *mg.* | *mg.* | *mg.* |
| Monitor H | 7 | 584 | 95.3 | 115.0 | 0.36 | 0.43 |
| Monitor H | 7 | 595 | 199.8 | 127.3 | 0.74 | 0.47 |
| Monitor H | 8 | 660 | 87.8 | 73.5 | 0.29 | 0.25 |
| Achiever H | 14 | 933 | 201.8 | 221.0 | 0.48 | 0.52 |
| Achiever H | 14 | 944 | 102.6 | 102.8 | 0.24 | 0.24 |
| Achiever H | 15 | 1009 | 83.7 | 54.6 | 0.08 | 0.05 |

males. It was decided to feed this animal 8 grams of chlorobutanol per day in accordance with the work reported by Sheidenhelm *et al.* (22) which showed that this compound would increase sexual activity. After one month the animal would serve a cow almost immediately. A second collection period of five days was made after the chlorobutanol treatment, and the ascorbic acid excretion was markedly increased as shown in table 4. The plasma and whole blood ascorbic acid values were also increased as had previously been noted with the normally fed females receiving chlorobutanol.

The results of a five-day collection period on male calves which had been continuously on definitely low carotene intakes since four months of age are shown in table 5. The carotene intake of this group varied from 22 to 40 micrograms per kilo of body weight. This difference in intake is reflected in the plasma vitamin A and carotene values. On the other hand, there does not appear to be any correlation between these levels of vitamin A intake and the excretion of ascorbic acid or blood ascorbic acid values. The values obtained are comparable to the normally fed animals as shown in tables 3 and 4.

TABLE 4

*Urinary excretion of ascorbic acid, blood ascorbic acid, vitamin A and carotene values of males fed a normal ration*

| | Day of collection | | | | | |
|---|---|---|---|---|---|---|
| | 1st | 2nd | 3rd | 4th | 5th | 6th |
| **Achiever H—1285 lbs.—Age, 1 yr. 10 mo.** | | | | | | |
| Ascorbic acid excreted, mg. | 423.2 | 304.6 | 476.0 | 319.2 | 304.2 | 308.2 |
| Excreted per kilo per day, mg. | 0.72 | 0.52 | 0.82 | 0.55 | 0.52 | 0.53 |
| Plasma ascorbic acid, mg./100 ml. | 0.36 | ..... | ..... | 0.29 | ..... | ..... |
| Whole blood ascorbic acid, mg./100 ml. | 0.65 | ..... | ..... | 0.64 | ..... | ..... |
| Plasma vitamin A, μg./100 ml. | 18.0 | ..... | ..... | ..... | ..... | ..... |
| Plasma carotene, μg./100 ml. | 118.0 | ..... | ..... | ..... | ..... | ..... |
| **Monitor H—1100 lbs.—Age, 1 yr. 2 mo.** | | | | | | |
| Ascorbic acid excreted, mg. | 104.2 | 182.3 | 181.3 | 138.4 | 147.9 | 106.2 |
| Excreted per kilo per day, mg. | 0.21 | 0.36 | 0.36 | 0.28 | 0.30 | 0.21 |
| Plasma ascorbic acid, mg./100 ml. | 0.23 | ..... | ..... | ..... | ..... | ..... |
| Whole blood ascorbic acid, mg./100 ml. | 0.61 | ..... | ..... | 0.48 | ..... | ..... |
| Plasma vitamin A, μg./100 ml. | 16.5 | ..... | ..... | ..... | ..... | ..... |
| Plasma carotene, μg./100 ml. | 70.0 | ..... | ..... | ..... | ..... | ..... |
| **467H—1060 lbs.—Age, 1 yr. 10 mo.** | | | | | | |
| Ascorbic acid excreted, mg. | 104.8 | 144.6 | 280.2 | 165.7 | 248.6 | 171.6 |
| Excreted per kilo per day, mg. | 0.22 | 0.30 | 0.58 | 0.34 | 0.52 | 0.36 |
| Plasma ascorbic acid, mg./100 ml. | 0.27 | ..... | ..... | 0.27 | ..... | ..... |
| Whole blood ascorbic acid, mg./100 ml. | 0.59 | ..... | ..... | 0.52 | ..... | ..... |
| Plasma vitamin A, μg./100 ml. | 16.7 | ..... | ..... | ..... | ..... | ..... |
| Plasma carotene, μg./100 ml. | 118.0 | ..... | ..... | ..... | ..... | ..... |
| **Sam J—1360 lbs.—Age, 7 yrs.** | | | | | | |
| Ascorbic acid excreted, mg. | 78.1 | 129.0 | 92.8 | 103.4 | 116.10 | 60.9 |
| Excreted per kilo per day, mg. | 0.13 | 0.21 | 0.15 | 0.17 | 0.19 | 0.10 |
| Plasma ascorbic acid, mg./100 ml. | 0.29 | ..... | ..... | 0.21 | ..... | ..... |
| Whole blood ascorbic acid, mg./100 ml. | 0.61 | ..... | ..... | 0.53 | ..... | ..... |
| Plasma vitamin A, μg./100 ml. | 22.4 | ..... | ..... | ..... | ..... | ..... |
| Plasma carotene, μg./100 ml. | 112.0 | ..... | ..... | ..... | ..... | ..... |
| **Sam J—1360 lbs.—Age, 7 yrs.—Fed chlorobutanol** | | | | | | |
| Ascorbic acid excreted, mg. | 270.7 | 193.1 | 154.9 | 199.9 | 165.3 | ..... |
| Excreted per kilo per day, mg. | 0.44 | 0.31 | 0.25 | 0.32 | 0.27 | ..... |
| Plasma ascorbic acid, mg./100 ml. | ..... | ..... | 0.45 | ..... | 0.41 | ..... |
| Whole blood ascorbic acid, mg./100 ml. | ..... | ..... | 0.97 | ..... | 0.60 | ..... |
| Plasma vitamin A, μg./100 ml. | ..... | ..... | ..... | ..... | 27.3 | ..... |
| Plasma carotene, μg./100 ml. | ..... | ..... | ..... | ..... | 152.0 | ..... |

Two-day collections were also made on a second group of four male calves fed carotene at definite but higher levels of intake than the previous group. This group had likewise been on the same carotene intakes since four months of age and the data collected are shown in table 6. The excretion of ascorbic

acid was comparable to the previous groups of males irrespective of carotene intake. The alfalfa meal fed the four calves as a source of carotene was discontinued for six weeks to bring the animals nearer to a severe state of

TABLE 5

*Urinary excretion of ascorbic acid, blood ascorbic acid, plasma vitamin A and carotene values of males on various low carotene intakes*

| | Day of collection | | | | |
|---|---|---|---|---|---|
| | 1st | 2nd | 3rd | 4th | 5th |
| **487H—Age, 1 yr. 1 mo.—670 lbs.—22 μg. carotene per kilo body weight** | | | | | |
| Ascorbic acid excreted, mg. | 166.3 | 188.2 | 123.1 | 130.0 | 125.9 |
| Excreted per kilo per day, mg. | 0.55 | 0.62 | 0.40 | 0.43 | 0.41 |
| Plasma ascorbic acid, mg./100 ml. | ..... | ..... | 0.21 | ..... | 0.27 |
| Whole blood ascorbic acid, mg./100 ml. | ..... | ..... | 0.82 | ..... | 0.63 |
| Plasma vitamin A, μg./100 ml. | ..... | ..... | ..... | ..... | 3.6 |
| Plasma carotene, μg./100 ml. | ..... | ..... | ..... | ..... | 8.0 |
| **482H—Age, 1 yr. 2 mo.—563 lbs.—26 μg. carotene per kilo body weight** | | | | | |
| Ascorbic acid excreted, mg. | 219.9 | 169.1 | 186.2 | 165.4 | 119.5 |
| Excreted per kilo per day, mg. | 0.86 | 0.66 | 0.73 | 0.65 | 0.47 |
| Plasma ascorbic acid, mg./100 ml. | ..... | ..... | 0.28 | ..... | 0.33 |
| Whole blood ascorbic acid, mg./100 ml. | ..... | ..... | 0.78 | ..... | 0.58 |
| Plasma vitamin A, μg./100 ml. | ..... | ..... | ..... | ..... | 7.5 |
| Plasma carotene, μg./100 ml. | ..... | ..... | ..... | ..... | 29.0 |
| **483H—Age, 1 yr. 2 mo.—655 lbs.—30 μg. carotene per kilo body weight** | | | | | |
| Ascorbic acid excreted, mg. | 101.0 | 99.2 | 96.9 | 96.0 | 97.2 |
| Excreted per kilo per day, mg. | 0.34 | 0.33 | 0.33 | 0.32 | 0.33 |
| Plasma ascorbic acid, mg./100 ml. | ..... | ..... | 0.28 | ..... | 0.28 |
| Whole blood ascorbic acid, mg./100 ml. | ..... | ..... | 0.75 | ..... | 0.52 |
| Plasma vitamin A, μg./100 ml. | ..... | ..... | ..... | ..... | 7.5 |
| Plasma carotene, μg./100 ml. | ..... | ..... | ..... | ..... | 32.0 |
| **486H—Age, 1 yr. 2 mo.—475 lbs.—36 μg. carotene per kilo body weight** | | | | | |
| Ascorbic acid excreted, mg. | 94.7 | 63.0 | 63.7 | 66.0 | 88.3 |
| Excreted per kilo per day, mg. | 0.44 | 0.29 | 0.30 | 0.31 | 0.41 |
| Plasma ascorbic acid, mg./100 ml. | ..... | ..... | 0.31 | ..... | 0.27 |
| Whole blood ascorbic acid, mg./100 ml. | ..... | ..... | 1.00 | ..... | 0.64 |
| Plasma vitamin A, μg./100 ml. | ..... | ..... | ..... | ..... | 8.1 |
| Plasma carotene, μg./100 ml. | ..... | ..... | ..... | ..... | 26.0 |
| **488A—Age, 1 yr. 1 mo.—534 lbs.—40 μg. carotene per kilo body weight** | | | | | |
| Ascorbic acid excreted, mg. | 194.4 | 191.8 | 190.6 | 109.6 | 130.2 |
| Excreted per kilo per day, mg. | 0.77 | 0.76 | 0.77 | 0.43 | 0.52 |
| Plasma ascorbic acid, mg./100 ml. | ..... | ..... | 0.28 | ..... | 0.38 |
| Whole blood ascorbic acid, mg./100 ml. | ..... | ..... | 0.65 | ..... | 0.56 |
| Plasma vitamin A, μg./100 ml. | ..... | ..... | ..... | ..... | 9.3 |
| Plasma carotene, μg./100 ml. | ..... | ..... | ..... | ..... | 36.0 |

vitamin A deficiency. The vitamin A and carotene values reflected this change in the ration as shown in table 6. Two of the calves were then fed three grams of clorobutanol for ten days before the next collection period.

TABLE 6

*Urinary excretion of ascorbic acid, plasma ascorbic acid, vitamin A and carotene values of males on various low carotene intakes*

| Animal No. | Age | Weight | Ascorbic acid excreted per day | | Ascorbic acid excreted per day per kilo | | Plasma ascorbic acid | | Plasma vitamin A | Plasma carotene | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1st day | 2nd day | 1st day | 2nd day | 1st day | 2nd day | | | |
| | *mo.* | *lbs.* | *mg.* | *mg.* | *mg.* | *mg.* | *mg./100 ml.* | *mg./100 ml.* | *µg./100 ml.* | *µg./100 ml.* | |
| 474H | 13 | 420 | 75.5 | 83.1 | 0.40 | 0.44 | ..... | ..... | 9.0 | 25.0 | 44* |
| 474H | 13 | 426 | 119.2 | 118.1 | 0.62 | 0.61 | ..... | ..... | 9.0 | 34.0 | 44* |
| 472G | 13 | 485 | 106.7 | 117.3 | 0.49 | 0.53 | ..... | ..... | 10.8 | 40.0 | 62* |
| 472G | 13 | 468 | 118.2 | 118.4 | 0.55 | 0.55 | ..... | ..... | 9.6 | 29.0 | 62* |
| 466A | 16 | 600 | 170.1 | 192.0 | 0.62 | 0.70 | ..... | ..... | 13.2 | 55.0 | 70* |
| 466A | 16 | 600 | 104.3 | 119.8 | 0.38 | 0.44 | ..... | ..... | 10.2 | 58.0 | 70* |
| 467H | 16 | 678 | 168.0 | 202.6 | 0.55 | 0.66 | ..... | ..... | 12.9 | 73.0 | 75* |
| 467H | 16 | 662 | 90.5 | 167.6 | 0.30 | 0.56 | ..... | ..... | 10.8 | 73.0 | 75* |
| Low carotene ration plus 3 gm. chlorobutanol per day | | | | | | | | | | | |
| 466A | 17 | 630 | 99.7 | 82.1 | 0.35 | 0.29 | 0.27 | 0.23 | 3.0 | 12.0 | |
| 467H | 17 | 733 | 301.9 | 322.0 | 0.91 | 0.97 | 0.34 | 0.33 | 3.3 | 10.0 | |
| Low carotene ration | | | | | | | | | | | |
| 472G | 14 | 492 | 46.2 | 38.3 | 0.21 | 0.17 | 0.22 | 0.23 | 1.8 | 7.0 | |
| 474H | 14 | 426 | 50.4 | 60.7 | 0.26 | 0.31 | 0.14 | 0.20 | 1.8 | 3.0 | |

* µg. carotene received per kilo per day.

The data show that one calf, 467H, was stimulated to excess excretion by the clorobutanol while 466A was not. Both calves showed the effects of the deficiency since they were both night blind and were not eating well. Animal 466A showed some diarrhea during the collection period.

The results obtained with the other two calves showed some depression of ascorbic acid excretion. Both calves were night blind and 472G had a convulsion during the collection period. Apparently, in extreme vitamin A deficiency, ascorbic acid excretion is decreased. The plasma ascorbic acid values also were somewhat depressed, a condition which has previously been noted in this laboratory in severely deficient calves.

The results of the experiments reported in this paper indicate that pregnant heifers on low carotene intakes (44 micrograms carotene per kilo body weight) excrete abnormally large amounts of ascorbic acid while non-pregnant heifers on the same ration excrete normal amounts when compared to normally fed animals. Normally fed males show considerable variation in the amount of ascorbic acid excreted. Male calves on restricted levels of carotene intake excreted normal amounts of ascorbic acid. It is significant that these calves had been on the low carotene ration for nine to ten months before the collections were made. Only when the calves were rendered extremely deficient in vitamin A was the amount of excretion of ascorbic acid depressed.

The results are not in harmony with those of Boyer *et al.* (4) and Sutton and co-workers (24) who used rats, except where extreme vitamin A deficiency was produced. It is possible that the calves fed on the restricted levels of carotene reported in this paper were not as deficient as the rats used by Boyer (4) and Sutton (24).

However, these data raise the question of whether or not, under practical conditions, the vitamin A intake is not always sufficient to prevent any marked depressing effect in ascorbic acid synthesis and thus indirectly affect reproduction. It does not seem likely that cattle under practical feeding conditions would ever remain on as low a level of carotene intake for any period of time as the animals reported in these experiments. For instance, if the carotene content of the hay being fed was only 10 micrograms per gram which is lower than No. 3 hays generally run (1), a 1000-lb. animal would have to consume only four to six pounds of hay per day in order to have an intake of 40 to 65 micrograms of carotene per kilo of body weight per day. This intake would be below that required for the production of normal calves. The production of blind and paralyzed calves on farms because of vitamin A deficiency is not a common occurrence. Kuhlman and Gallup (11) have pointed out that a satisfactory conception rate was obtained when the carotene intake was at the same level as that required for normal calving.

On the other hand, it is not impossible that high intakes of vitamin A

or carotene above that received in normal winter rations might stimulate ascorbic acid synthesis and increase plasma ascorbic acid values above normal or above the average values of animals on winter rations. The possibility of this effect should be further investigated.

It seems probable that in extreme deficiency the synthesis and metabolism of many substances might be affected. Sure (23) noted lowered plasma ascorbic acid values in rats on diets deficient in vitamins A, $B_1$ and riboflavin. Phillips (19) pointed out that the riboflavin content of the liver of the vitamin A deficient animals as well as the plasma ascorbic acid values was low. It would seem questionable, therefore, that the effect of a low vitamin A intake was specific in disturbing ascorbic acid synthesis.

No attempt was made to correlate plasma ascorbic acid values and urinary excretion of ascorbic acid. Bortree *et al.* (2) has pointed out that random plasma ascorbic acid determinations are of little value in determining the normal concentration of this vitamin in the blood of dairy animals. In order to show any correlation it would be necessary to draw frequent blood samples during the collection period. There does appear to be some correlation between excretion and plasma ascorbic acid values in the case of the normally fed heifers which received chlorobutanol. Under these circumstances there was an increase in both values. In the case of the severely deficient male calves, there was a decrease of both values.

## SUMMARY

1. Ascorbic acid excretion in normally fed heifers varied from 25.8 to 110.5 mg. per day.

2. When fed 5 grams of chlorobutanol for 10 days normally fed heifers excreted 210 to 399 mg. of ascorbic acid per day.

3. Ascorbic acid excretion of pregnant heifers receiving a ration low in carotene was from 4 to 6 times greater than in similar animals normally fed.

4. The feeding of 5 grams of chlorobutanol for 10 days to pregnant heifers receiving a ration low in carotene stimulated the excretion of ascorbic acid very little.

5. The chlorobutanol caused an increase in plasma ascorbic acid values of the normally fed pregnant heifers but not in similar heifers receiving a low carotene ration.

6. Dairy males excreted varying amounts of ascorbic acid regardless of the level of carotene intake, except when made severely deficient, in which case there was a decrease in ascorbic acid excretion.

7. Normally fed males excreted considerably more ascorbic acid per kilo of body weight than normally fed females.

8. It still remains to be shown that a low carotene intake under practical farm conditions has a depressing effect on ascorbic acid synthesis to the extent of altering breeding efficiency.

## REFERENCES

(1) Baltzer, A. C., Huffman, C. F., and Duncan, C. W. The Chemical Composition of Michigan Grown Hays as Fed to Dairy Cattle. I. The Carotene Content. Mich. Agr. Expt. Sta. Quart. Bul., 25: 8–B. 1942.

(2) Bortree, A. L., Huffman, C. F., and Duncan, C. W. Normal Variations in the Amount of Ascorbic Acid in the Blood of Dairy Cattle. Jour. Dairy Sci., 25: 983–990. 1942.

(3) Bortree, A. L., Huffman, C. F., and Duncan, C. W. Ascorbic Acid Stimulation in the Blood Plasma of Dairy Cattle Produced by the Ingestion of Chlorobutanol. Jour. Dairy Sci., 26: 553–562. 1943.

(4) Boyer, P. D., Phillips, P. H., Pounden, W. D., Jensen, C. W., Rupel, I. W., and Nesbit, M. E. Certain Relationships of Avitaminosis A to Vitamin C in the Young Bovine. Jour. Nutr., 27: 525–531. 1942.

(5) Golden, W. R. C., and Garfinkel, L. Medical Evaluation of Nutritional Status. XII. The Stability of Ascorbic Acid in Whole Blood Plasma and Plasma Filtrates. Jour. Biol. Chem., 144: 447–452. 1942.

(6) Hart, G. H., Goss, H., and Guilbert, H. R. Vitamin A Deficiency not the Cause of Joint Lesions in Horses. Amer. Jour. Vet. Res., 4: 162–168. 1943.

(7) Jensen, C., Boyer, P. D., Phillips, P. H., Rupel, I. W., and Lundquist, N. S. The Effect of Shark Liver Oil on Milk and Butter Fat Production. Jour. Dairy Sci., 25: 931–937. 1942.

(8) Jones, I. R., Haag, J. R., and Brandt, P. M. Growth Reproduction and Lactation of Dairy Cattle Fed Dry Rations Varying in Mineral and Vitamin Contents. Oreg. Agr. Exp. Sta. Bul., 329. 1934.

(9) Jones, I. R., Haag, J. R., and Daugherty, R. W. The Relation of Nutrition to Breeding Performance in Dairy Bulls. Jour. Dairy Sci., 25: 689–690. 1942.

(10) Kimble, M. S. The Photoelectric Determination of Vitamin A and Carotene in Human Plasma. Jour. Lab. and Clin. Med., 24: 1055–1065. 1939.

(11) Knight, C. A., Dutcher, R. A. and Guerrant, N. B. Utilization and Excretion of Ascorbic Acid by the Dairy Cow. Jour. Dairy Sci., 24: 567–577. 1941.

(12) Kuhlman, A. H., and Gallup, W. D. Carotene Requirements of Dairy Cattle for Conception. Jour. Dairy Sci., 25: 688–689. 1942.

(13) Longnecker, H. E., Fricke, H. H., and King, C. G. The Effect of Organic Compounds upon Vitamin C Synthesis in the Rat. Jour. Biol. Chem., 135: 497. 1940.

(14) Mindlin, R. L., and Butler, A. M. The Determination of Ascorbic Acid in Plasma. Jour. Biol. Chem., 122: 673–678. 1938.

(15) Moore, L. A. The Determination of Blood Plasma Carotene in the Bovine Using a Photoelectric Colorimenter. Jour. Dairy Sci., 22: 501–511. 1939.

(16) Moore, L. A., Berry, M. H., and Sykes, J. F. Carotene Requirments for the Maintenance of a Normal Spinal Fluid Pressure in Dairy Calves. Jour. of Nutr., 26: 649–658. 1943.

(17) Musulin, R. R., Tully, R. H., 3rd, Longnecker, H. E., and King, C. G. Vitamin C Synthesis and Excretion by the Rat. Jour. Biol. Chem., 129: 437. 1939.

18) Phillips, P. H., Boyer, P. D., Lardy, H. A., and Lundquist, N. S. Vitamin A Levels in the Blood Plasma of Dairy Cattle on Winter Rations and the Influence of Vitamin A Supplementation on Certain Constituents of the Blood. Jour. Dairy Sci., 24: 522. 1941.

(19) Phillips, P. H., Rupel, I. W., and Olsen, J. J. The Effects of Atypical Blindness-Producing Ration upon the Vitamin K and C Content of Calf Blood. Amer. Soc. Anim. Prod. Proc., 320–327. 1938.

(20) Roe, J. H., and Kuether, C. A. The Determinations of Ascorbic Acid in Whole Blood and Urine Through the 2,4-Dinitro-phenylhydrazine Derivative of Dehydro-ascorbic Acid. Jour. Biol. Chem., **147**: 399–407. 1943.

(21) Rubin, M., and Bird, H. R. Ascorbic Acid Storage in Vitamin A Deficient Hens. Poultry Sci., **22**: 53–55. 1943.

(22) Scheidenhelm, E. C., Bortree, A. L., Huffman, C. F., and Clark, C. F. Some Preliminary Results of Feeding Chloretone to Bulls. Jour. Dairy Sci., **25**: 690. 1942.

(23) Sure, B., Theis, R. M., and Harrelson, R. T. Vitamin Interrelationship. 1. Influence of Avitaminosis on Ascorbic Acid Content of Various Tissues and Endocrines. Jour. Biol. Chem., **129**: 245. 1939.

(24) Sutton, T. S., Kaeser, H. E., and Hansard, S. L. Some Factors Affecting the Synthesis of Ascorbic Acid in the Albino Rat. Jour. Biol. Chem., **144**: 183–191. 1942.

(25) Wallis, G. C. Evidence of the Synthesis of Vitamin C by Dairy Cows. Jour. Dairy Sci., **26**: 401–408. 1943.

## NOTE FROM THE EDITOR

This note is to call attention to a Special Reprint on the preparation of Scientific Papers from the Transactions of the American Association of Cereal Chemists. This reprint consists of a series of 6 papers covering the subject.

I am sure that many research workers find the preparation of scientific papers a difficult task. Few have had formal training in this specific field, and textbooks or other useful publications on the subject are not numerous nor widely disseminated. The need is great. In recognition of this need, the Association of Cereal Chemists planned and published this series of reports.

Inasmuch as these papers contain many suggestions that may prove helpful to the contributors of the JOURNAL OF DAIRY SCIENCE, I recommend it to you. Reprints may be obtained by writing to H. L. Harris, Managing Editor, Association of Cereal Chemists, University Farm, St. Paul 8, Minnesota. The price of a single copy is 50 cents.

EDITOR

VOLUME XXVIII JUNE, 1945 NUMBER 6

# JOURNAL OF DAIRY SCIENCE

Published by the
AMERICAN DAIRY SCIENCE ASSOCIATION
R. B. STOLTZ, Sec.-Treas.
Ohio State University, Columbus, Ohio

# ABSTRACTS OF LITERATURE

T. S. SUTTON, Editor
Columbus, Ohio

## MILK AND MILK PRODUCTS

Published in cooperation with
INTERNATIONAL ASSOCIATION OF ICE CREAM MANUFACTURERS
R. C. HIBBEN, 1105 Barr Bldg., Washington, D. C., Exec. Sec.

INTERNATIONAL ASSOCIATION OF MILK DEALERS
R. E. LITTLE, 309 W. Jackson Blvd., Chicago, Illinois, Exec. Sec.

*Editorial Committee*

H. H. SOMMER, Madison, Wisconsin, A. D. S. A.
G. D. TURNBOW, Oakland, California, I. A. I. C. M.
HAROLD PRATT, Philadelphia, Pennsylvania, I. A. I. C. M.
A. J. POWERS, Brooklyn, New York, I. A. M. D.
V. CHRISTIANSEN, Chicago Illinois, I. A. M. D.

CONTENTS

*Bacteriology*
*Breeding*
*Butter*
*Cheese*
*Chemistry*
*Concentrated and dry milk; by-products*
*Diseases*
*Feeds and feeding*
*Food value of dairy products*
*Herd management*
*Ice cream*
*Milk*
*Miscellaneous*
*Physiology*

THE SCIENCE PRESS PRINTING COMPANY LANCASTER, PA.

# PUBLICATIONS AND ABSTRACTORS

## EDITORS

Dahle, C. D., Dahlberg, A. C., Horrall, B. E., Petersen, W. E., Tracy, P. H. and Weckel, K. G.

## ABSTRACTORS

Archibald, J. G.
Bennett, F. W.
Berggren, Ruth E.
Burgwald, L. H.
Burkey, L. A.
Bushnell, L. D.
Call, A. O.
Cole, W. C.
Coulter, S. T.
Doan, F. J.
Dorsey, L. M.
Downs, P. A.
Ely, Fordyce
Erb, J. H.
Espe, D. L.
Frazier, W. C.
Fuller, S. A.
Glick, D. P.
Goss, E. F.
Henderson, J. L.
Huffman, C. F.
Irvine, O. R.
Josephson, D. V.
Lucas, P. S.
Lush, J. L.
Marquardt, J. C.
Mueller, W. S.
Price, W. V.
Pyenson, Harry
Reece, Ralph P.
Roberts, Henry E.
Thomsen, L. C.
Trimble, C. S.
Trout, G. M.
Waugh, R. K.
Webb, B. H.
Weckel, K. G.
Weiser, H. H.
White, G. C.
Yale, M. W.

## JOURNALS

American Butter Review
American Milk Review
American Journal of Diseases of Children
American Journal of Physiology
American Journal of Public Health
Archives of Pediatrics
Australian Journal of the Council for Scientific and Industrial Research

Biochemical Journal

Canadian Dairy and Ice Cream Journal
Canadian Journal of Public Health
Canadian Journal of Research
Certified Milk
Cornell Veterinarian

Dairy Industries
Dairy World

Endocrinology

Food in Canada
Food Industries
Food Manufacture
Food Research

Ice and Refrigeration
Ice Cream Field
Ice Cream Review
Ice Cream Trade Journal
Industrial and Engineering Chemistry

Journal of Agricultural Research
Journal of Agricultural Science
Journal of American Medical Association
Journal of American Veterinary Medical Association
Journal of Animal Science
Journal of Bacteriology
Journal of Biological Chemistry
Journal of Dairy Research
Journal of Dairy Science
Journal of Endocrinology
Journal of Experimental Medicine
Journal of General Physiology
Journal of Genetics
Journal of Heredity
Journal of Infectious Diseases
Journal of Milk Technology
Journal of Nutrition
Journal of Pathology and Bacteriology
Journal of Physical Chemistry
Journal of Physiology
Journal of Veterinary Research

Lancet
Le Lait

Milk Dealer
Milk Industry
Milk Plant Monthly

National Butter and Cheese Journal
New Zealand Journal of Science and Technology

Oil and Soap

Pacific Dairy Review
Proceedings of Society of Experimental Biology and Medicine

Refrigerating Engineering

Scientific Agriculture
Southern Dairy Products Journal

## SPECIAL PUBLICATIONS

Federal Dairying and Bacteriological Establishment, Liebefeld, Berne, Switzerland

International Association of Ice Cream Manufacturers
International Association of Milk Dealers

National Institute for Research in Dairying, Reading, England
New York Association of Dairy and Milk Inspectors

Prussian Dairy Research Institute, Kiel, Germany

State Agricultural Colleges and Experiment Stations

The Royal Technical College, Copenhagen, Denmark

United States Department of Agriculture

# ABSTRACTS OF LITERATURE

## BACTERIOLOGY

**168. A Study of the Germicidal Power of Sodium Chloroorthophenylphenate Compared with Calcium Hypochlorite in the Presence of Tomato Juice.** W. A. NOLTE AND L. H. JAMES, Dept. of Bact., Univ. of Maryland, College Park, Maryland. Food Res., *9*, No. 5: 367. Sept.–Oct., 1944.

One of the limitations of the commonly used chlorine compounds as disinfectants and sterilizers in dairy plants is the loss of bactericidal efficiency in the presence of small quantities of organic matter. In this study, sodium chloroorthophenylphenate, one of a group of newer germicides obtainable from Dow Chemical Company, was found to have high killing power (99 per cent) even in a solution containing 2.77 per cent solids (tomato) at a concentration of 0.025 per cent. Calcium hypochlorite (a good germicide in the absence of organic matter) had no bactericidal action under like conditions at concentrations between 40 p.p.m. and 254 p.p.m. of available chlorine and between pH 4.60 and 6.07. F.J.D.

**169. A New Modification of the Frost Little Plate for the Detection of Heat Resistant Bacteria in Milk.** C. S. BRYAN AND A. L. BORTREE, Michigan Agr. Expt. Sta., East Lansing, Mich. Jour. Milk Tech., *7*, No. 5: 251. Sept.–Oct., 1944.

A modification of the Frost little plate technic, which is both rapid and accurate is presented for the detection of heat-resistant bacteria in milk. The new method was compared with the standard laboratory pasteurization test on 1375 samples of milk and was found to check favorably. The advantages of this method are saving in materials and time. Results are available in five hours instead of 48 hours by the standard method.

Following is the modified technic:

1. Place a test tube rack, containing enough sterile tubes for the number of samples to be tested, into a water bath at 45° to 50° C.
2. Place 1.0 ml. of milk into a sterile test tube in the water bath. Do not allow more than 15 minutes between step 2 and completing step 4.
3. Add 1.0 ml. of sterile tryptone-glucose-extract agar, cooled to 50° C., into each tube in the water bath, and mix by shaking.
4. Using a sterile pipette place 0.1 ml. of the agar-milk mixture onto a sterilized microscope slide (sterilized in the naked flame) and spread evenly over an area of 4 square centimeters with the tip of the pipette. (Two or three such films can be put on the ordinary microscope slide.)
5. Place the tube containing the milk-agar mixture into a water bath at pasteurizing temperature for ½ hour.

6. Remove to a 50° C. water bath and prepare a second little plate immediately (this will give the living bacteria count after pasteurization).

7. The little plate is allowed to harden and then placed into a moist chamber at 37° C. for a minimum of *four hours of incubation for milk and cream.* The incubation period may be extended to 24 hours without materially affecting the bacteria count. A moist chamber can be made out of any container with a tight-fitting cover by filling the container approximately ¾ full of water. A wire platform must be built above the level of water to hold the slides during incubation. The proper temperature of bath is maintained by leaving the moist chamber in the 37° C. incubator at all times.

8. After icubation the plates are dried over a flame, in an incubator, or in a drying oven at a temperature slightly under 100° C. (Agar should not melt; plates should be dried slowly to prevent cracking the medium.)

9. Stain for approximately 1 minute with a methylene blue solution. (To prepare, add 10 ml. of a saturated alcoholic solution to 90 ml. of 30 per cent alcohol.) This should stain the colonies deeply and leave a faint blue background.

10. Wash slides to remove excess stain, being careful not to wash the film off the slides. (Best results are obtained by allowing the water to strike the reverse side of slide—enough water will flow over the preparation to remove the excess stain.) Dry preparations.

11. Examine by using low power (if few colonies), high dry power or oil immersion (if many colonies) to determine the average number of colonies per field (count 50 fields when few colonies are present, and 10 fields when many colonies are present).

A table is given for quickly estimating the count. L.H.B.

**170. A Microscopic Field Spacer.** T. C. Buck, Jr., Asst. Dir., Bur. of Laboratories, Baltimore City Health Dept., Baltimore, Md. Jour. Milk Tech., *7*, No. 6: 341. Nov.–Dec., 1944.

A description and illustration of the apparatus is given.

It is considered that the use of the spacer for Breed counts on milk has considerable value as a time saver. L.H.B.

**171. The Incidence and Types of Coliform Organisms in Pasteurized Cream.** Elinor Van Dorn Smith, Dept. of Hyg. and Bact., Smith Col., Northampton, Mass. Jour. Milk Tech., *7*, No. 6: 338. Nov.–Dec., 1944.

In a study of 178 samples of cream received from a health laboratory following their tests and kept in an ice box for 2 to 7 days during the winter of 1942, coliform organisms were isolated from 111 samples or 62.3%, using

1-m. inoculations in brilliant green lactose peptone bile broth. Sixty-seven samples or 37.6% were "coliform" satisfactory.

Of the 178 samples studied, 18 were raw, 12 were marked pasteurized but were found to be underpasteurized by the phosphatase test, 148 were pasteurized.

On the 148 pasteurized samples, 57 samples or 38.5% were "coliform" satisfactory, while 91 samples or 61.5% yielded coliform organisms.

Of the 91 samples which yielded coliform organisms on the confirmation test, six were negative on the presumptive test.

Of the 57 samples, which were considered "coliform" satisfactory, ten were positive on the presumptive test, but negative on the confirmation test.

Of the 111 coliform positive samples, 24.3% contained *Escherichia*, 71% contained *Aerobacter* and 63% contained intermediate forms. L.H.B.

**172. A Comparison of Two Standard Media for the Detection and Enumeration of *Escherichia* and *Aerobacter* in Milk.** C. E. SKINNER, RICHARD MARWIN, AND O. HONAAS, Dept. of Bacteriology and Immunology, Univ. of Minn., Minneapolis, Minn. Jour. Milk Tech., *7*, No. 4: 200. July–Aug., 1944.

Some comparative tests of brilliant green-lactose-peptone-bile broth and sodium formate-sodium ricinoleate-lactose-peptone broth for determining the efficiency in detecting *Escherichia-Aerobacter* bacteria in raw milk were made on 111 samples.

No evidence was found that either medium gives significantly more false negative tests than the other.

The formate-ricinoleate broth gave significantly more false positive tests than did the brilliant green bile broth. The majority of these false positive tests were due to species of *Proteus* and a few were due to *Salmonella*.

L.H.B.

## BREEDING

**173. Influence of Inbreeding upon the Weight and Size of Dairy Cattle.** S. MARGOLIN AND J. W. BARTLETT, N. J. Agr. Expt. Sta., Rutgers, N. J. Jour. Anim. Sci., *4*, No. 1: 3–12. Feb., 1945.

In this experiment inbreeding did not cause a decrease in body weight or size at any stage, from birth to maturity, as compared with the outbred controls of the same blood line, provided the Wright coefficient of inbreeding did not exceed 0.20. Females inbred to a coefficient greater than 0.20 developed normally to approximately first calving age, but showed markedly adnormal development thereafter.

The progeny of the Holstein Friesian foundation cattle were mated as follows: a) sire-daughter matings; b) brother-sister matings; c) matings with less than the same blood; and d) outbreeding. The last system of mating has furnished adequate controls for the inbreeding investigations.

The results reported are a confirmation and an extension of the earlier reports of the New Jersey Station which have established that inbreeding can be applied practically to the breeding of dairy cattle, provided superior foundation animals are used and rigid selection is employed. C.F.H.

**174. Differences in Frequency of Cellular Antigens in Two Breeds of Dairy Cattle.** R. D. OWEN, C. J. STORMONT, AND M. R. IRWIN, Dept. of Genetics, Univ. Wis., Madison. Jour. Anim. Sci., *3*, No. 4: 315–321. Nov., 1944.

Frequencies of 30 cellular antigens in a sample of 569 Guernseys were compared with those of 513 Holstein-Friesian cows. Eighteen of the antigens were significantly more frequent in Guernseys; seven were not significantly different in frequency in the two breeds, while 5 were significantly more frequent in Holsteins. No single antigen by itself distinguishes these two breeds. The breed differences in blood type are the result of quantitative differences in frequencies of most of the antigens. Although the computation of simple gene frequencies cannot be accomplished until the genetic associations between antigens are better established, there is little doubt that the breed differences in antigen frequency are the result of contrasting gene frequencies among genes common to the two breeds. Each breed appears to be potentially capable under selection of producing individuals identical in blood type with members of the other breed. C.F.H.

## CHEESE

**175. Extraneous Matter Tolerances in Cheese.** WALTER V. PRICE AND RAYMOND MIERSCH, Univ. of Wis., Madison, Wis. Jour. Milk Tech., *7*, No. 6: 322. Nov.–Dec., 1944.

The application of a "control chart" system for inspecting the quality of cheese is justified on the basis that it is unreasonable to expect perfection in cheese sediment tests.

Steps for the analytical laboratory to follow in setting up and using the quality control chart are given.

Instructions are also given for setting up tentative standards.

The authors' conclusions are as follows: "Our observations over a three-year period indicate that:—the elimination of excessive amounts of extraneous matter from cheese is practical, and feasible; that the reduction of contamination of cheese with critical extraneous matter, like hairs, is possible; and that these two objectives can be accomplished without unduly increasing costs of manufacture. Regulations requiring absolute elimination of all critical extraneous matter must be enforced with tolerance until better production methods can be demonstrated as feasible and economical under commercial conditions or until convincing proof is offered that the public health is in danger." L.H.B.

## CHEMISTRY

**176. An Application of the Barcroft-Warburg Apparatus to the Study of Antioxidants in Fats.** J. J. NAGY, F. C. VIBRANS, AND H. R. KRAYBILL, Res. Lab., Am. Meat Inst., Chicago, Ill. Oil and Soap, *21*, No. 12: 349. Dec., 1944.

The testing of antioxidants in fats by three procedures, (1) active oxygen, (2) Barcroft-Warburg apparatus at 70° C. and (3) storage at room temperatures, demonstrated the fact that when evaluating new substances for antioxidant properties different conclusions may be drawn, depending upon the procedure used. The data show that the experimental condition of the test should not be very different from the conditions under which the antioxidant is to be used. The importance of correlating the results of accelerated tests with storage tests is emphasized.

The three methods gave results that were in good agreement with lard to which no antioxidant had been added or to lard to which lecithin alone was added. With lard to which d-isoascorbyl palmitate or d-isoascorbyl monsterate (0.01 to 0.1 per cent) is added, the results with the Barcroft-Warburg apparatus are in much better agreement with the storage tests than are those by the active oxygen method. The d-isoascorbyl esters in concentrations of 0.01 to 0.1 per cent in lard were found to usually behave as antioxidants with the active oxygen method and as pro- or antioxidants, depending on their concentration, with the Barcroft-Warburg apparatus or with the storage test at room temperature. J.L.H.

## CONCENTRATED AND DRY MILK; BY-PRODUCTS

**177. Powdered Whole Milk and Milk Products—Their Possible Effect upon the Future of the Dairy Industry.** P. H. TRACY, Dept. of Dairy Husb., Univ. of Ill., Urbana, Ill. Jour. Milk Tech., *7*, No. 4: 206. July–Aug., 1944.

One of the limiting factors in the acceptance of powdered whole milk is its tendency to develop a stale, oxidized flavor. Progress has been made in this connection, however, and it is predicted that oxidation will cease to be a major problem after this war is over. Some of the factors, which will lessen the tendency toward the development of oxidized flavor, are mentioned.

A number of other factors, which will effect sales or consumer acceptance of powdered milk are convenience and relative cost.

The enthusiasm of the industry and the research that will be conducted to improve the quality of the products will also be very important factors. L.H.B.

## DISEASE

**178. Mastitis and the Plate Count of Milk. III. A Quantitative Study of the Growth of *Streptococcus uberis* in Various Plating Media.** H. W. SEELEY, JR., E. O. ANDERSON, AND W. N. PLASTRIDGE, Storrs Agr. Expt. Sta., Storrs, Conn. Jour. Milk Tech., *7*, No. 4: 191. July–Aug., 1944.

In order to find a suitable media for the differential determination of *Str. uberis* in plated samples, fifteen strains of *Str. uberis* were plated in parallel in each of standard agar, ox-blood agar, and Edwards' crystal-violet aesculin ox-blood agar.

The identities of the cultures employed were checked both biochemically and serologically.

"Although blood agar yielded greater numbers of colonies than either of the other two media, it does not have properties which permit the differentiation of *Str. uberis* colonies from those of other bacteria found in the mixed flora of milk. Edwards' agar, besides having the advantage of being equal to standard agar in growth-supporting ability for *Str. uberis,* permits some degree of preliminary identification of these colonies, and for these reasons offers relatively good possibilities as a plating medium for this organism." L.H.B.

**179. Mastitis and the Plate Count of Milk. Part IV. The Contribution of *Streptococcus uberis* Mastitis to the Plate Count of Herd Milk.** H. W. SEELEY, JR., E. O. ANDERSON, AND W. N. PLASTRIDGE, Storrs Agr. Expt. Sta., Storrs, Conn. Jour. Milk Tech., *7*, No. 4: 195. July–Aug., 1944.

In this study milk from 23 quarters of animals known to be infected with *Str. uberis* were plated on standard medium and on Edwards' medium which contained mannite, inulin and sorbitol substituted for aesculin which war conditions had made impossible to purchase. *Str. uberis* is characterized by a greening of the medium adjacent to the colony on this medium.

Plate counts of *Str. uberis* ranged from 30 to 44,000 per ml. in this study. There is evidence, however, that under certain conditions, quarters infected with *Str. uberis* may release much larger numbers of this organism in the milk; as in one instance cited, the number of organisms ranged from 700,000 to 1,420,000 per ml. in milk from six quarters in one herd.

"Under the conditions of this experiment *Str. uberis* counts obtained from infected quarters were not great enough to influence the plate count of high-count milk. In some instances, as in a small herd, or in a herd in which the incidence of *Str. uberis* is high, organisms released from the infected animals might materially increase the total plate count."

L.H.B.

**180. Mastitis and the Plate Count of Milk. V. The Behavior of *Streptococcus uberis* in Milk Held at Different Temperatures.** H. W. SEELEY, JR., E. O. ANDERSON, AND W. N. PLASTRIDGE, Storrs Agr. Expt. Sta., Storrs, Conn. Jour. Milk Tech., *7*, No. 6: 329. Nov.–Dec., 1944.

A study was made to note the effect the presence of *Str. uberis* might have on the total bacterial count and the acidity of milk under different conditions of cooling.

The rate of growth and lactose-fermenting activity of fifteen strains of *Str. uberis* were measured at 40°, 50°, 60°, 70°, and 97° F. over a 60-hour period.

The results indicated that cooling and storage temperatures of 50° F. or below effectively prevent growth of *Str. uberis* and a change in pH even for 60 hours.

Milk held at 60° F. for 12 to 24 hours causes a fairly rapid increase in the numbers of *Str. uberis*, and for longer periods it is perceptibly greater. At higher temperatures the increase is rapid.

No appreciable change in pH was noted at 60° F. until after 48 hours. At 70° F. no appreciable change was noted until after 36 hours. An appreciable change was noted after 12 hours at 70° F.

The results indicated that the presence of *Str. uberis* mastitis in a herd may contribute materially to the total plate count of the milk from that herd if the milk is cooled and stored at temperatures above 50° F.

A statistical analysis of the experimental data is given. L.H.B.

**181. A Cheese-Borne Epidemic of Typhoid Fever.** JACQUES GAUTHIER, Director, Champlain County Health Unit, AND A. R. FOLEY, Prov. Epidemiol., Min. of Health and Social Welfare, Quebec, Canada. Jour. Milk Tech., *7*, No. 4: 211. July–Aug., 1944.

(Reprinted from the Canadian Journal of Public Health, Dec., 1943.)

L.H.B.

## FEEDS AND FEEDING

**182. On the Mechanism of Non-Protein-Nitrogen Utilization by Ruminants.** B. C. JOHNSON, T. S. HAMILTON, W. B. ROBINSON, AND J. C. GAREY, Univ. of Ill., Urbana, Ill. Jour. Anim. Sci., *3*, No. 3: 287–298. Aug., 1944.

Defaunated growing lambs utilize the nitrogen of urea-containing rations to about the same extent as normal lambs. The average digestibility of nitrogen in a ration in which 83 per cent of the total nitrogen was in the form of urea, was 68.4 and the biological value 49. Rumen protozoa and bacteria were separated in a fairly high state of purity. The dried protozoa contained 54.7 per cent crude protein, which had true digestibility of 86 per cent and a biological value of 68. The bacterial fraction from the rumen

contained 44.5 per cent when dried while a bacterial fraction obtained by culture on synthetic media contained 58.8 per cent protein. The digestibility of this protein was 82.4 per cent and the biological value was 66.

Methane production was highest during the first hour following feeding. There was a greater number of bacteria and fewer protozoa one hour after feeding. A yeast-like organism was observed in the rumen in very large numbers.

The data are in agreement with the hypothesis that in ruminants, much of the food nitrogen, whether of protein or non-protein nature, is first synthesized by the bacteria into their own cellular proteins and then, at least to a considerable extent, the protozoa utilize bacterial protein in their own growth, and finally the host digests the protozoal protein; and remaining bacterial protein. The biological value of the protein from bacteria and protozoa seems to approximate 60. Nitrogen consumed above that required by the microorganisms should exhibit a biological value approximating that of a non-ruminant of similar requirements. C.F.H.

**183. Studies on the Carotenoid and Vitamin A Levels in Cattle. I. Seasonal Changes of the Carotenoid and Vitamin A Levels and the Normal Carotenoid-Vitamin A Ratio of the Blood.** W. BRAUN, Div. Vet. Sci., Univ. Calif., Berkeley. Jour. Nutr., *29*, No. 1: 61–71. Jan., 1945.

The carotenoid and vitamin A levels of the blood of 49 cows maintained in four dietary groups with different carotenoid and vitamin A intakes, were determined periodically for one year.

The seasonal changes of the carotenoid level were mainly dependent on the carotenoid intake, but varied according to the age and breed of the animal. Pathological disturbances showed no direct effect on the carotenoid level.

The seasonal changes of the vitamin A level were dependent on carotenoid and vitamin A intakes. The vitamin A levels showed a sharp temporary decrease during parturition, abortion and acute infections. The vitamin A level rose immediately after administration of shark liver oil.

There was a linear increase of vitamin A with increasing carotenoid levels. The A/car. ratio decreased with increased carotenoid levels reaching a constant value at high carotenoid levels. The A/car. ratio was below normal in parturition, abortion and acute infections. C.F.H.

**184. Studies on the Carotenoid and Vitamin A Levels in Cattle. II. Carotenoids and Vitamin A in the Liver, Their Ratio and Their Relationship to Blood Levels.** W. BRAUN, Div. Vet. Sci., Univ. Calif., Berkeley, Calif. Jour. Nutr., *29*, No. 1: 73–79. Jan., 1945.

The carotene and vitamin content of the blood plasma and livers of cows fed various levels of carotene and vitamin was determined.

The vitamin A levels of the liver were significantly different among three of the four dietary groups, but similar for the shark liver oil supplemented groups regardless of their basic diet. This indicated that there is an optimum level for vitamin A storage. The results obtained from livers of animals which had been maintained on a vitamin A enriched diet 6 to 8 months previously indicated a rather lasting effect on the vitamin A storage from a relatively short period of vitamin A feeding. The results with livers from vitamin A starved animals and on samples obtained by partial hepatectomy, utilization of stored vitamin A first forces available carotenoid stores to be converted into vitamin A, thus decreasing the carotenoid level without decreasing the vitamin A level. Similarly to the A/car. ratios observed in the blood, a typical relationship between carotenoid levels and corresponding vitamin A levels appeared to exist in the liver. The A/car. ratio of the liver was modified if the animal was fed vitamin A. In certain pathological conditions a tendency towards a direct relationship between vitamin A stores and the vitamin A level of the blood existed only when the former fell below normal levels. C.F.H.

## FOOD VALUE OF DAIRY PRODUCTS

**185. Digestibility of Milk as Affected by Various Types of Treatment.** A. W. TURNER, Dept. of Physiological Chemistry, Univ. Ill., Col. of Med. and Chem. Res. Lab., Armour and Co., Chicago, Ill. Food Res., *10*, No. 1: 52. Jan.–Feb., 1945.

Milk digestibility was studied *in vitro* using both acid and alkaline digestion periods in the presence of enzymes from the hog stomach, duodenum and pancreas. The index of digestibility was taken as the rate of breakdown of the "acid-insoluble proteins" which were measured by adjusting the digest to a pH of 4.7, after varying periods of digestion, filtering and determining the nitrogen content of the filtrate.

The results obtained by this method showed no effect on digestibility when milk was pasteurized, homogenized, desiccated, treated with zeolite or skimmed. Heat-treated and enzyme-treated milks digested relatively faster than pasteurized milk. Mare's milk digested more readily than cows' milk while goat's milk digested less rapidly. Barium sulfate added to milk prevented the normal digestion of the milk proteins. F.J.D.

**186. Preliminary Studies on Stabilization of Thiamin in Dehydrated Foods.** E. E. RICE, J. F. BEUK, F. L. KAUFFMAN, H. W. SCHULTZ, AND H. E. ROBINSON, Nutrition Div., Res. Lab., Swift and Co., Chicago, Ill. Food Res., *9*, No. 6: 491. Nov.–Dec., 1944.

Dehydrated eggs and pork were found to lose thiamin much more rapidly, when stored at 37° C. (98.6° F.), than certain other foods such as dried milk

or cereals. Thiamin retention in dehydrated pork was greatly improved when the dried milk or cereals were added to the pork (67% pork and 33% milk or cereals). The improved stability of the thiamin appeared to be caused by materials rich in carbohydrates. F.J.D.

**187. The Nicotinic Acid, Pantothenic Acid, Choline and Biotin Content of Fresh, Irradiated Evaporated and Dry Milk.** A. Z. HODSON, Pet Milk Co., Greenville, Ill. Jour. Nutr., *29*, No. 2: 137–142. Feb., 1945.

The average nicotinic acid, pantothenic acid, choline and biotin content of fresh milk was found to be 0.91 mg., 3.1 mg., 149 mg. and 47.1 micrograms per liter respectively.

There were no significant losses in the content of nicotinic acid, pantothenic acid, choline and biotin in the processing of irradiated evaporated, dry skim or dry whole milk. The sources of the milks used in the determination of the above vitamins varied. C.F.H.

## MILK

**188. The Trumbull Electro-Pure Pasteurizer.** A. M. PALMER, Trumbull Electric Mfg. Co., Plainville, Conn. Jour. Milk Tech., *7*, No. 5: 283. Sept.–Oct., 1944.

The new Trumbull Electro-Pure outfit is made in sizes from 150 gallons to 900 gallons per hour. The units are complete and in addition to the pasteurizer include a plate regenerator and plate cooler. The pasteurizer uses a constant rate of flow; an automatic electrical temperature control, and a flow diversion valve.

A complete description together with diagrams of the unit are given. L.H.B.

**189. Short-Time-High-Temperature Pasteurization.** R. E. OLSON, Mgr. Sales Engin. Dept., Taylor Instrument Companies, Rochester, N. Y. Jour. Milk Tech., *7*, No. 5: 276. Sept.–Oct., 1944.

The importance of automatic temperature control equipment and its proper maintenance is stressed. A description and diagram of a typical short-time pasteurizer control system is given. The proper care and operation of the automatic control system are discussed as are some of the causes of unsatisfactory performances and means for correcting them. L.H.B.

**190. A Northeastern States Code for Milk for Pasteurization.** WALTER D. TIEDEMAN, N. Y. Dept. of Health, Albany, N. Y. Jour. Milk Tech., *7*, No. 5: 270. Sept.–Oct., 1944.

A group of representatives from eight states formulated a set of simpli-

fied regulations for the production of raw milk for pasteurization. The regulations are modifications of the U. S. Public Health Service ordinance.

The adoption of this system would have the effect of overcoming many inconsistencies in the present programs of sanitary control within this area. It would prevent arguments about conflicting farm requirements or procedures by adjacent health agencies. It would prevent a slovenly dairyman excluded from one market to find another market for his milk. It would make it easier to prevent the transfer of diseased cattle from one dairy herd to another. It would make it easier for many of the dairymen in the area to meet essential requirements and establish stable markets for quality milk.

There are twenty-five items in this simplified code pertaining to the production and transportation of milk. Bacterial standards are also given.

L.H.B.

**191. Cleaning and Sterilizing Short-Time Pasteurizers.** C. M. MOORE, Dir. of Res., The Diversey Corporation, Chicago, Ill. Jour. Milk Tech., *7*, No. 5: 291. Sept.–Oct., 1944.

Inasmuch as higher temperatures are used for pasteurizing milk in short-time pasteurizers, and also the fact that a great volume in milk is handled over relatively small areas of equipment, the cleaning job is more difficult than for other equipment.

The type of milk deposit to be removed when 165° F. is used as compared to 144° F. is quite different in composition. The rate of heating is also a factor; slow heating gives a different type of deposit than fast heating. For steam-heated units double treatment is recommended, first with an acid-base milkstone remover and then with an alkaline milkstone remover. For electric type units only the special acid-base milkstone remover is necessary.

Complete instructions for cleaning procedure, which has proven satisfactory in hundreds of plate-type pasteurizing units in all types of hard-water condition, are given; as are instructions for sterilizing plate-type units.

L.H.B.

**192. Variability in Quality of Cotton Lintine Disks Used for Determining Extraneous Material in Milk.** K. G. WECKEL, Dept. of Dairy Indus., Univ. of Wis., Madison, Wis. Jour. Milk Tech., *7*, No. 5: 260. Sept.–Oct., 1944.

Considerable variations were found in thickness and distribution of cotton within the disk and in irregularity of cut.

The weights of sediment test disks in one box had a maximum variation (based on the lowest value) of 19.10%.

The thickness of disks at random indicated that nearly every box contained some disks having a definite irregularity in diameter such as being somewhat oval in shape instead of circular. Deviation amounted to as much as 6 millimeters in some instances.

It is advised that attention should be given to attaining greater uniformity in the quality of sediment test disks. L.H.B.

**193. The Laboratory Control of Added Water to Milk. A Discussion of the Cryoscopic Method.** M. A. NUSSBAUM, St. Bd. of Health, Jackson, Miss. Jour. Milk Tech., *7*, No. 5: 255. Sept.–Oct., 1944.

The practice of watering milk has been increasing during our present emergency and it is advised that the frequent and intelligent use of an official quantitative method for determining the presence of added water will have a tendency to halt this adulteration. For this purpose the cryoscopic method is both reliable and practical for routine use.

The principle of this method is discussed as is the method for standardizing the thermometer and calculating of results. L.H.B.

**194. Steam High-Temperature-Short-Time Pasteurizers.** DALE GILLESPIE, The Creamery Package Mfg. Co., Chicago, Ill. Jour. Milk Tech., *7*, No. 5: 287. Sept.–Oct., 1944.

A description and flow diagram of the system are shown.

Ordinarily high-temperature-short-time pasteurizers are not as adaptable for very small capacities. Clean-up time for a small unit is practically the same as for a large unit. Also, the longer the run, the more economical the outfit would be, as here again the clean-up time would be the same.

No set formula is available for determining whether or not a high-temperature-short-time system should be installed. A thorough study of each individual operation must be made.

Important pointers on operation and maintenance are given. L.H.B.

**195. A Study of Methods for the Microscopic Examination of Raw Milk with Suggested Improvements.** W. L. MALLMANN AND C. S. BRYAN, Dept. of Bact., AND W. D. BATEN, Dept. of Math., Mich. Agr. Expt. Sta., East Lansing, Mich. Jour. Milk Tech., *7*, No. 6: 315. Nov.–Dec., 1944.

Comparisons were made on raw milk samples using Standard Methods pipette technic, 0.01 ml. of milk smeared over 1.0 sq. cm., and Bryan's loop technic, using a 4-mm. (outside diameter) loop of milk smeared over a $4 \times 8$ mm. surface.

When results were analyzed statistically, it was found that the Bryan loop technic was equally as accurate as the Standard Method pipette technic.

Comparisons also were made using a modification of the staining method recommended in Standard Methods and an acid stain of Mallmann and Churchill.

The modified Standard Methods staining procedure used was as follows:

Immerse slides in xylene for about one minute, drain, and immerse in 95% alcohol, for one minute, drain and immerse in staining bath prepared

by adding 10 ml. of saturated methylene blue to 90 ml. of 30% alcohol; just long enough for proper staining. They are then rinsed in water and decolorized in alcohol, if necessary, followed by thorough drying.

The acid stain of Mallmann and Churchill is prepared by mixing 1 gm. of methylene blue (certified for bacteriological use); 500 ml. of 95% ethyl alcohol; and 0.5 to 5.0 ml. of concentrated hydrochloric acid (the greater the amount the clearer the background).

The slides after immersing in xylene are then immersed in this staining bath for 3 to 5 minutes (alcohol bath is omitted.) They are then dipped in a tap water bath only long enough to remove excess stain. Do not decolorize entirely as it will make it difficult to focus the microscope or to detect the edges of the smear. The slides are air-dried.

In this comparison all smears were made by the loop technic.

The acid stain yielded higher counts on 31 of 40 samples of raw milk, and afforded more ease and speed in counting.

An orange filter (Wratten M filter No. E–22) instead of a blue filter commonly used between the light source and the object is recommended.

It is suggested that an accuracy of 25% be required for producer milk samples. It is also suggested that the accuracy be appended as plus or minus per cent of that use, depending upon the number of fields counted. Example, where the average number of organisms per field is approximately 4 and an accuracy of 5% is desired, it would be necessary to count 137 fields. While if the average number of organisms per field is approximately 0.4 for a 5% accuracy, it would be necessary to count 1250 fields. For an accuracy of 25% with 4 organisms per field, it would be necessary to count only 6 fields, and with 0.4 organism per field, it would be necessary to count 50 fields for a 25% accuracy.

It is recommended that the Bryan loop technic and the Mallmann and Churchill acid stain be offered as an alternative procedure for standard methods for the examination of raw milk. L.H.B.

**196. Quality Control in the Dairy Industry.** ERNEST C. THOMPSON, The Borden Co., 350 Madison Ave., New York 17, N. Y. Jour. Indus. Engin. Chem., Indus. Ed., *37*, No. 3: 208. Mar., 1945.

The accomplishments of the dairy industry in the field of quality control during the last 50 years are reviewed. Diseased cattle and attendants have been removed from their milk production functions. Important steps in sanitary control have been introduced. The meaning of the quality control program in the manufacture of butter, ice cream, cheese, condensed, evaporated and dry milks is discussed. Composition and consumer-acceptance factors are presented for major milk products. Quality control is being used in the fields of standardization, fortification and nutrition. B.H.W.

## PHYSIOLOGY

**197. Changes in the Physiology and Pharmacology of the Uterine Muscle of the Cow in Relation to the Estrous Cycle.** P. T. CUPPS AND S. A. ASDELL, Lab. of Anim. Nutr., Cornell Univ., Ithaca, N. Y. Jour. Anim. Sci., *3*, No. 4: 351–359. Nov., 1944.

Excised uterine muscle of cattle killed at known stages of the estrous cycle was used. The results showed that spontaneous motility is greatest during proestrum and estrous, becomes irregular during metestrum and dies down during diestrum. Pituitrin, arecolin and lentin produced strong tonic contractions at all stages of the cycle. Estrine, gynergen and pilocarpine were mainly inert. Atropine rarely produced an inhibitory effect. Epinephrin was inhibitory during and shortly after the estrogenic phase, and motor during the progestational phase.

The muscle cells of the uterus increased in length during the estrogenic phase and decreased in length during the rest of the cycle. C.F.H.

**198. The Action of Adrenaline and of Choline-Esters on the Uterus of the Sheep.** J. A. GUNN. Nuffield Institute for Medical Research, Oxford. Jour. Physiol., *103*, No. 3: 290. Dec., 1944.

Adrenaline and epinephrine stimulate the contraction of exsected non-pregnant uteri of sheep and goats but inhibit those of pregnant sheep (goats not studied). Tyramine, B-phenylethylamine, acetylcholine and carbachol stimulate the contractions of both pregnant and non-pregnant uteri.

D.E.

## MISCELLANEOUS

**199. Post-War Planning on Dairy Equipment.** A. W. FARRALL, Dir. of Res., The Creamery Package Mfg. Co., Chicago, Ill. Jour. Milk Tech., *7*, No. 5: 264. Sept.–Oct., 1944.

The following points are considered in this discussion: 1. Increased demand for equipment. The need for replacement will be at least 50% greater than normal. 2. Quality improvements in dairy products. The fact that the dairy industry and public health agencies are interested in improving the quality of dairy products both from the flavor as well as from the health standpoint. Dairy machinery is continually being improved with this in mind. 3. Processing efficiency. Having machinery which will do the jobs more efficiently. Butter, for instance, will be manufactured by a continuous process. 4. Improvements in materials and fabrications will be a certainty. More use of stainless steel and other corrosion-resistant alloys. Plastics will, undoubtedly, be used for small parts. 5. Technical operators will be more in demand, men who are well trained

in the fundamentals of dairy technology and engineering so that they will get the best results from plant equipment. L.H.B.

**200. A Course of Instruction for Food Handlers.** A. A. ROBERTSON, Chief Sanitary Officer, Health Dept., Newton, Mass. Jour. Milk Tech., 7, No. 6: 349. Nov.–Dec., 1944.

Four illustrated lectures have been prepared on the following subjects: 1. Disease Transmission from the Standpoint of Food Handling. 2. Sanitization of Eating and Drinking Utensils. 3. Care of the Establishment and Inspection and Care of Food. 4. Personal Hygiene and Service.

Each lecture is about one hour in length, and the number permitted to attend a lecture is limited to 25. A true-false test is given at the end of each lecture.

A certificate is given all persons attending the first lecture and any two of the other three.

A description of the material used in each lecture is given. L.H.B.

**201. Softening Water for Use in Dairy Plants.** C. E. LENNOX, Mgr., Equip. Div., Klenzade Products, Inc., Chicago 50, Ill. Jour. Milk Tech., 7, No. 6: 343. Nov.–Dec., 1944.

The availability of soft water greatly simplified boiler operations and the cleaning problems in a dairy plant.

Practically all water requires some treatment to suit it to the requirements of the process it is to be used for.

Hard water troubles encountered in a dairy plant which are discussed are: boiler operation, hot water supplies, can washing, bottle washing, water-cooling coils, equipment cleaning, and washroom maintenance.

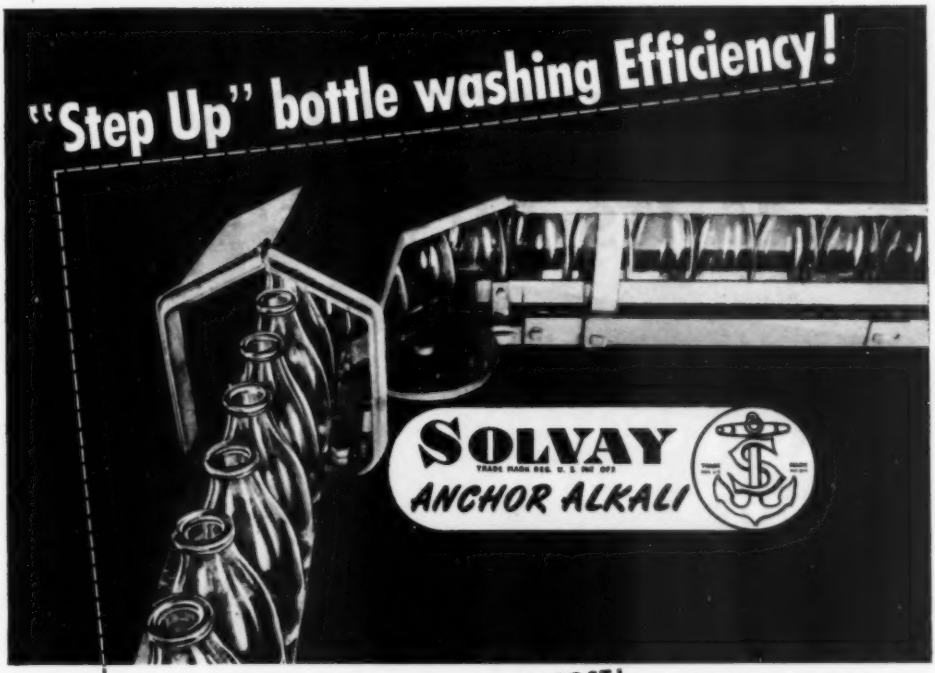
The importance of pre-treatment of water is discussed, as are some of the processes for accomplishing it, such as the hot lime-soda process, the cold lime-soda process, the use of siliceous zeolites and the carbon type exchangers.

Where completely pre-treated waters are not available, corrective measures, such as the selection of cleaning materials and methods that will give the least trouble in operating must be resorted to.

The various phosphate compounds are considered to be the best "water softeners" among the alkaline materials.

Acid cleansers, a mixture of organic acids and wetting agents, are good for correcting water for hardness and may be better for some cleaning jobs than an alkaline compound. L.H.B.

# "Step Up" bottle washing Efficiency!



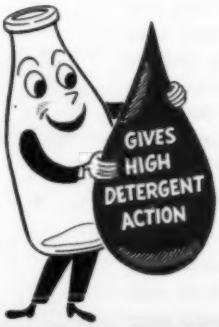

## ---GET CLEANER BOTTLES AT LESS COST!

You can "step up" bottle washing efficiency—and save both time and money! It's *easy* with Solvay Anchor Alkali. Here is the PROVED scientifically efficient bottle washing compound—*at work* daily in dairy plants throughout the country.

Comparative tests prove that its high detergent action produces brighter, cleaner, sterile bottles *quicker*. Anchor Alkali is in dustless flake form, easy to handle—dissolves completely, at least ⅓ faster than most other alkalies—gives uniform strength—rinses free. It's economical, too, because less of it is required to wash a given number of bottles in both hard and soft waters.

Try Anchor Alkali. *See* and *time* the difference in bottle washing efficiency—and save on lower over-all cost.

SEND IN COUPON TODAY for complete folder which tells you how Anchor Alkali is used.




for maximum
bottle washing efficiency...
maximum economy...
use Solvay Anchor Alkali

**SOLVAY SALES CORPORATION,** 40 Rector St., New York 6, N. Y.

*Gentlemen:* Kindly send me your complete folder describing Anchor Alkali for use in dairies.

*Name*.....

*Affiliated with*.....

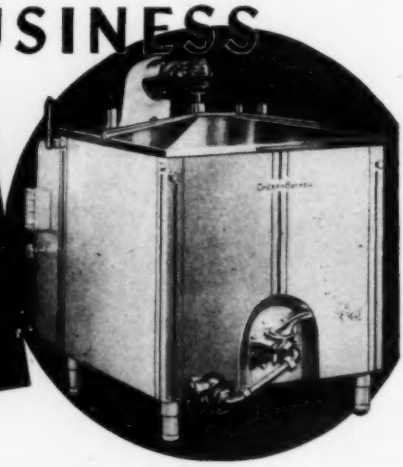
*Address*.....

*City*..... *State*..... CX-6

**Your advertisement is being read in every State and in 25 Foreign Countries**

# VERSATILITY IS ITS BUSINESS

*The* SPRAY-F PASTEURIZER DOES MANY JOBS



If you own a SPRAY-F PASTEURIZER and have kept it in good condition — or if you have the proper priorities which will permit us to supply you a new one — you're in luck!

★

HOW ABOUT ANOTHER $100.00 WAR BOND TODAY?

★

For a SPRAY-F is a machine that does many jobs and does them all *well*. With it you can pasteurize, cool, hold and store milk — make buttermilk, starter and ice cream mix — make chocolate milk and other similar dairy products. And, what is more, your SPRAY-F PASTEURIZER needs no "extras" — it is completely equipped with start and stop switches, built-in connections, built-in sanitary milk pump, hot water pump and motors, leak detector flush valve and sanitary fittings.

★

★

Some SPRAY-F's are available, on proper priorities, in both the 100 and 150-gallon sizes and insulated as well as non-insulated models. Discuss your requirements soon with your Cherry-Burrell representative.

CHERRY-BURRELL CORPORATION

*Specialists in Equipment and Supplies for the Dairy Industry*

427 WEST RANDOLPH STREET, CHICAGO 6, ILLINOIS

FACTORIES, WAREHOUSES, BRANCHES, OFFICES OR DISTRIBUTORS AT YOUR SERVICE IN 52 CITIES

Your advertisement is being read in every State and in 25 Foreign Countries

# How much are two and two?

"Tu eres mi caballo, Plato, and a very *lucky* horse. You do not pull the plow. You do not draw the cart. You have the life of ease. I often wonder if you know how much are two and two."

"But yes, Don Miguel, I have often *seen* the teams of horses and the teams of oxen. Now, I tell you something: They pull together. One helps the other to do more work, better work. There is no limit to what two and two can do!"

"Caramba! Qué una cabeza! Teamwork is what we have here at Hacienda Miguel. Nature and Science, they pull together as a team, for one is really part of the other. Nature, she gave to the Mayas and Aztecs the wonderful vanilla plant of the orchid family.

"But we, of David Michael, we rescue the wild plant from its native jungle and transplant it in our vanillery at Puerto Rico. There we coil it upon slender supports where it can have the right balance of sun and shade. We add the chemical elements to the soil for healthier progress."

"When you gotta grow, you gotta grow."

"Some day you choke on the joke! But to continue. We assist Madre Nature to produce finer and finer vanilla beans. But of what value are they without our expert curing? And in the Michael laboratory, in Philadelphia, they are scientifically processed and enhanced with the vanillin derived from tropical spice oils. Una otra vez, we have Nature and Science putting two and two together. This vanillin, mi caballo, she is the key to fine vanilla flavor. But what do you suppose is the most important ingredient?"

"I'll bite."

"So, you'll bite, and Don Miguel will kick out your beautiful white teeth! The ingredient más importante is the David Michael *experience*. That, Plato, is what produces *controlled flavor* in Michael's Mixevan . . . and the world's finest vanilla ice cream. You learn something to-day, Plato?"

"Si, señor. A closed mouth catches no flies. As we say it: 'En boca cerrada, no entran moscas.'"

"Exactamente! So that people will eat more ice cream, they must be told the story of all these things."

*Mixevan—"America's Flavorite"*
*and other powdered vanilla products*

**DAVID MICHAEL & CO.**

*half a century in the flavoring field*

**Front and Master Streets, Philadelphia 22, Pa.**

Our bulletin 30c is fully descriptive—Write for it.

"THE GAULIN TWO STAGE HOMOGENIZER AND HIGH PRESSURE PUMPS have met the Dairy Industries needs for a durable, heavy duty and economical machine, since 1909. Homogenizers and Pumps are still available with proper priority ratings, as well as repair and replacement parts. Both machines are strictly sanitary and are approved by all leading health boards. The Homogenizers are built in sizes from 75 gallons to 2000 gallons per hour, and the High Pressure Pumps are custom built to suit individual needs. The Gaulin is the ideal, all-purpose machine for fluid milk, ice cream, evaporated milk, condensed milk and spray-drying milk or eggs."

MANTON-GAULIN
TWO STAGE
HOMOGENIZER

THE MANTON-GAULIN MFG. CO., INC.
7 CHARLTON STREET EVERETT, MASS., U.S.A.

## QUICKER Way to Clean Milk Cans

For BETTER, FASTER can washing, try that quick-acting cleaning agent, OAKITE COMPOSITION No. 82. This specialized material does a thorough cleaning job . . . does it quickly and with safety to tinned surfaces. Moreover, it rinses readily . . . leaves cans clean, sanitary, odor-free!

And when it is necessary to brighten dull, dingy milk and cream cans, you can "spruce up" such equipment by using OAKITE COMPOUND No. 84. Through the dual combination of acidic properties and emulsifying action, rust film, hard water deposits and milkstone are thoroughly removed . . . cans come out clean and bright-looking . . . both inside and out. Let us send you FREE details!

OAKITE PRODUCTS, INC.,
Dairy Research Division
16G THAMES STREET, NEW YORK 6, N. Y.
Technical Service Representatives Located in All Principal Cities of the United States and Canada

OAKITE CLEANING

## NU-KLEEN

### ORGANIC DETERGENT

**For All Types of Equipment Cleaning by Hand**

Simplify your cleaning problem by using this new effective organic detergent especially balanced for all hand cleaning operations. NU–KLEEN is one of today's most popular "acid" cleaners. Its organic acid content is skillfully adjusted to the wetting agent concentration providing the ideal amount of penetration and coverage. NU–KLEEN removes and prevents milkstone. It is free rinsing and leaves no film or other deposits. You can brush or spray NU–KLEEN with equally good results and do away with laborious old time cleaning methods. NU–KLEEN is non-corrosive and harmless to hands and clothing. Keep a supply on hand always for plant use and for your patrons' milking utensils and milking machines.

KLENZADE PRODUCTS, INC.
Beloit, Wisconsin

Your advertisement is being read in every State and in 25 Foreign Countries

# TELL US MORE
## *Teacher!*

● Maybe you're like a lot of food technologists and salt buyers who have gone along thinking all salt is the same. Actually, of course, various brands and grades and grains of salt differ in many respects.

Take solubility: In salting cheese, slow solubility is highly important. Otherwise, salt is lost in the whey, producing flat, under-salted cheese. On the other hand, in salting butter, salt must dissolve with lightning speed. If the butterfat is on the soft side—lacking in body—at certain seasons, butter salt must dissolve so quickly that over-working is avoided. Otherwise, the butter may lose its desirable physical properties and become mottled or marbled—and may lose its moisture, become leaky. Yet, if the salt is not properly dissolved, the butter may be gritty.

To meet these problems, we have set up definite solubility standards for Diamond Crystal Salt. Our Butter Salt, for example, dissolves completely in water at 65° F. in less than 9.8 seconds — average rate, 9.2 seconds. That is why so many quality-minded food processors have learned to depend on Diamond Crystal products, manufactured under strict quality-control standards for solubility rate.

**Want Free Information On Salt? Write Us!**

If salt solubility enters into your processing, write to our Technical Director. He will gladly recommend the correct Diamond Crystal Salt for best results. Diamond Crystal, Dept. H-12, St. Clair, Michigan.

**DIAMOND CRYSTAL**
ALBERGER PROCESS **SALT**

## FLAV-O-LAC FLAKES

**THE CULTURE**

of definitely better flavor & aroma-producing qualities.

The standard with foremost operators, agricultural schools & colleges.

**FLAV-O-LAC FLAKES** (shown) produce a quart of the finest starter on a single propagation. Single bottles $2.00.

**SPECIAL FLAV-O-LAC FLAKES "40"** produce 40 quarts of starter on a single propagation. Single bottles $3.00

**Free Culture Manual of Fermented Milk Products on request.**

*Pioneers in Spectro-chemical, Chemical and Fluoro-photometric Determinations of Vitamins A, $B_1$, $B_2$, Nicotinic Acid, Pantothenic Acid, $B_6$, C & E in Dairy and Food Products. (Vitamin D excluded) inquiries invited.*

**THE DAIRY LABORATORIES**
23rd & Locust Sts., Phila., Pa.
DIVISION DALARE ASSOCIATES
BRANCHES
New York Baltimore Washington
See our catalog in Dairy Industries Catalog.

# STALEY'S

Corn Gluten Feed
Corn Gluten Meal
Corn Oil Meal
Soybean Oil Meal
Soybean Oil Meal Pellets

*Each Feed Made to Exacting High Standards*

## A. E. Staley Mfg. Co.

Decatur, Illinois — Painesville, Ohio

**Your advertisement is being read in every State and in 25 Foreign Countries**

## to the very last drop

When a Seal-Kap goes over the pouring lip of a clear glass bottle, it is there to protect the contents right through to the end.

During handling and delivery, the pouring lip is completely shielded from danger of contaminating contacts.

In the home, Seal-Kaps come off the bottle with an easy twist of the wrist, avoiding fork-prying and splashing hazards.

And tough, durable Seal-Kaps go back on, as often as necessary, with a tight-fitting snap. As long as there is milk in the bottle, it is safe from spilling and danger of adjacent food taints.

With Seal-Kap protection, pure milk stays pure, right down to the last drop. Now, more than ever, they guard the nation's vital milk supplies.

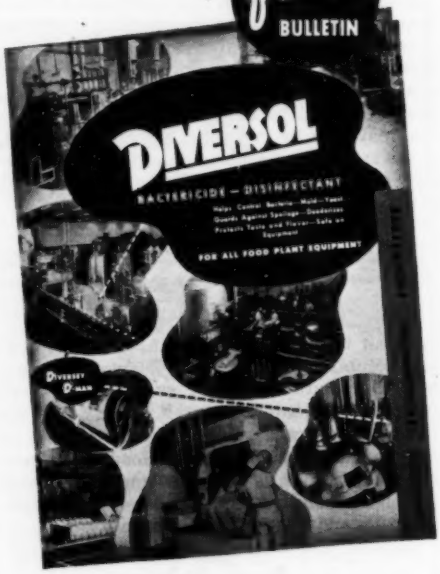
**AMERICAN SEAL-KAP CORPORATION**

11-05 44th Drive, Long Island City, N. Y.

*SEAL-KAP COVERS THE DANGER ZONE*

# 10 "Action" features revealed

**...IN THIS** *free* **BULLETIN**




**HERE'S *proof* BACTERICIDES ARE DIFFERENT!**

10 "action" features make the difference! Send for this free bulletin . . . see for yourself how and why Diversol is different from ordinary bactericides. A quick acting, crystal sodium hypochlorite, Diversol helps control bacteria, mold and yeast . . . guards against spoilage . . . helps protect taste and flavor. Softens hard water . . . leaves no film or scale. Send for your copy of this interesting bulletin today. **The Diversey Corporation, 53 W. Jackson Blvd., Chicago 4, Ill.**

**Your advertisement is being read in every State and in 25 Foreign Countries**

THE HOLSTEIN-FRIESIAN Association of America was established in 1885 for the purpose of improving the Holstein-Friesian breed of cattle, preserving and publishing the pedigrees and production records and promoting the best interests of the breed in general.

*But Our Interests Have Always Had a Much Wider Scope*

We have always been interested in contributing as much as possible to the general progress and improvement of the Dairy Industry as a whole through our program of breed improvement.

That is why our members recognized from the beginning (1885) the value of testing cows for production and why we adopted the Babcock test (in 1894) as a means of determining the amount of butterfat in milk. More than 69,000 production records have been recorded by Advanced Registry and more than 118,000 records have been completed in our Herd Test program to date.

That is why we recognized the need for and established (in 1923) the True Type or "ideal" Holstein cow and bull. That is why we established our Herd Inspection and Classification Program (in 1929) in which more than 27,000 animals have been given an official rating.

That is why we believe in the utmost cooperation with other purebred dairy cattle associations, with research and extension workers in colleges and experiment stations, and with dairymen's groups which are designed to assist in the progress of the Dairy Industry as a whole.

**THE HOLSTEIN-FRIESIAN ASSOCIATION OF AMERICA**
**BRATTLEBORO, VERMONT**

**Established in 1885** **33,000 Members**

**"HANSEN'S" DAIRY PREPARATIONS**

CHEESE RENNET AND COLOR
ANNATTO BUTTER COLOR
CERTIFIED BUTTER COLOR
ICE CREAM COLOR
LACTIC FERMENT CULTURE
BULGARIAN CULTURE

★

TESTING SOLUTIONS
RENNET TESTS

★

Chr. Hansen's Laboratory, Inc.
Milwaukee 14, Wisconsin

*Marschall*
**RENNET and COLOR**

For best results in the cheese vat, specify Marschall's. High in strength, pure and uniform.

**MARSCHALL**
**DAIRY LABORATORY, INC.**
**MADISON 3, WISCONSIN**

**Your advertisement is being read in every State and in 25 Foreign Countries**

# AMERICAN DAIRY SCIENCE ASSOCIATION

INCORPORATED IN THE DISTRICT OF COLUMBIA

### *Officers*

*President* ..... A. C. RAGSDALE, Columbia, Missouri
*Vice-President* ..... J. A. NELSON, Bozeman, Montana
*Secretary-Treasurer* ..... R. B. STOLTZ, Columbus, Ohio
*Editor* ..... T. S. SUTTON, Columbus, Ohio
*Director* ..... P. A. DOWNS, Lincoln, Nebraska
*Director* ..... J. A. LINN, Manhattan, Kansas
*Director* ..... P. H. TRACY, Urbana, Illinois
*Director* ..... C. L. BLACKMAN, Columbus, Ohio
*Director* ..... R. B. BECKER, Gainesville, Florida
*Director* ..... W. E. PETERSEN, St. Paul, Minnesota
*Director* ..... A. C. DAHLBERG, Ithaca, New York

### *Officers of Sections*

#### DAIRY PRODUCTION SECTION

*Chairman* ..... GLEN SALISBURY, Ithaca, N. Y.
*Vice-Chairman* ..... DWIGHT SEATH, University, La.
*Secretary* ..... GEORGE WISE, Clemson, S. C.

#### DAIRY MANUFACTURING SECTION

*Chairman* ..... L. K. CROWE, Lincoln, Nebraska
*Vice-Chairman* ..... C. L. HANKINSON, Milwaukee, Wis.
*Secretary* ..... N. S. GOLDING, Pullman, Washington

#### DAIRY EXTENSION SECTION

*Chairman* ..... FLOYD ARNOLD, College Park, Maryland
*Vice-Chairman* ..... W. T. CRANDALL, Ithaca, New York
*Secretary* ..... E. H. LOVELAND, Burlington, Vt.

### *Officers of Divisions*

#### SOUTHERN

*Chairman* ..... R. B. BECKER, Gainesville, Fla.
*Vice-Chairman* ..... J. P. LAMASTER, Clemson, S. C.
*Secretary-Treasurer* ..... F. H. HERZER, State College, Miss.

#### EASTERN

*Chairman* ..... H. C. MOORE, Durham, N. H.
*Vice-Chairman* ..... A. I. MANN, Storrs, Conn.
*Secretary-Treasurer* ..... L. R. DOWD, Storrs, Conn.

#### WESTERN

*Chairman* ..... N. S. GOLDING, Pullman, Wash.
*Vice-Chairman* ..... S. W. MEAD, Davis, Calif.
*Secretary-Treasurer* ..... H. P. EWALT, Corvallis, Oregon

*The American Dairy Science Association* was organized to advance the general welfare of the dairy industry, especially by the improvement of dairy instruction by the stimulation of scientific research in all phases of the subject and by improvement in methods of conducting extension work.

*Membership.* Any person is eligible to membership who is formally announced by an Agricultural College or Experiment Station, or by the Bureau of Dairy Industry of the United States Department of Agriculture or by the Canadian Department of Agriculture as an instructor, extension worker, investigator, or administrative officer connected with the dairy industry, or any person filling a position of responsibility connected with the dairy industry who has had a college or university training in technical science, or any person filling a responsible position in the dairy industry of a professional character requiring a technical knowledge of dairying of a high order. The membership fee is $5.00.

The dues are $5.00 a year, $3.00 of which is for a year's subscription to the *Journal of Dairy Science*. Correspondence regarding membership and dues should be addressed to R. B. Stoltz, Ohio State University, Columbus, Ohio.

# What you get when you use Patapar

## Wet-Strength Protection

Patapar* Vegetable Parchment is the hi-wet strength paper. Soaked in water—even boiled—it keeps its strength and beauty.

When a moist food is wrapped in it Patapar holds firm, and the product inside is kept fresh and appetizing.

## Resistance to grease

This same paper has the power to resist the penetration of grease, fats, oils.

That's why butter, cheese, poultry and other hard-to-protect products are wrapped in it. They keep better.

## Colorful printing

Patapar has a rich distinctive texture for printing in one or more colors. The printing is done right in our own plants where we have complete modern equipment—both letterpress and offset lithography.

## Keymark

*Reg. U.S. Pat. Off.

The Patapar Keymark is the nationally advertised symbol of protection. When you include it on your printed wrappers you tell your customers that your product is well protected.

**Paterson Parchment Paper Company • Bristol, Pennsylvania**

*Headquarters for Vegetable Parchment for 60 Years*

WEST COAST PLANT: 340 BRYANT STREET, SAN FRANCISCO 7, CALIFORNIA

BRANCH OFFICES: 120 BROADWAY, NEW YORK 5, N. Y. • 111 WEST WASHINGTON ST., CHICAGO 2, ILL.

**Your advertisement is being read in every State and in 25 Foreign Countries**

Still a most Efficient Economical Bactericide

B·K
REG. U.S. PAT. OFF.
CHLORINE BACTERICIDE

**B-K Powder combines speedy, thorough germicidal action with economy. It is widely distributed, is one of the best-known bactericides on the market and its application is generally understood by producers and plants alike. B-K Powder contains 50% available chlorine. It is easy to use, highly concentrated, an economical chlorine bactericide. Costs about one-sixth cent per gallon for 100 p.p.m. solution.**

**Our B-K Plan with B-K Chlorine Bactericide and General Manual Kleanser, together with helpful educational material, provides a complete service for Quality Programs. This service has been tested and proved by wide usage. We will gladly supply details and the material.**

**Write us for the B-K Plan.**

Dept. DS, BK Division
PENNSYLVANIA SALT MANUFACTURING COMPANY
Chemicals
1000 WIDENER BUILDING, PHILADELPHIA 7, PA.



Looking Forward

Wartime achievements in science are developing an era of progress which challenges the imagination.

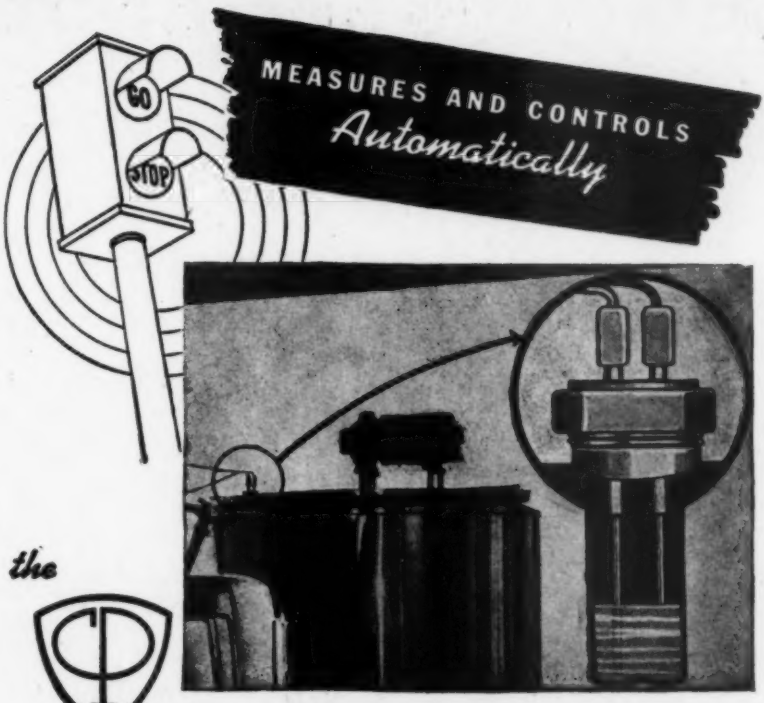
All that Spencer is doing now — producing microscopes, periscopes, telescopes, aircraft and anti-aircraft gunsights, prism binoculars, azimuth instruments for directing artillery fire, tank sights, telescopic alidades for navigation, projectors for instruction—will reap peacetime rewards in advanced knowledge, better manufacturing techniques, finer instruments.

At the war's end, Spencer will be ready to serve scientific optical needs on a far broader scale than ever before.

ARMY E NAVY

Spencer LENS COMPANY
BUFFALO, NEW YORK
SCIENTIFIC INSTRUMENT DIVISION OF
AMERICAN OPTICAL COMPANY


**Your advertisement is being read in every State and in 25 Foreign Countries**




MEASURES AND CONTROLS *Automatically*

*the*

# LACTRON LIQUID-LEVEL CONTROL

**Operates by electronics. No floats or moving parts to get out of order or complicate clean-up. Accurate to 1/1000 of an inch. Provides a safe, dependable and sanitary means for MEASURING and CONTROLLING the volume of any fluid product. Easily and quickly installed in vats or processing tanks, surface coolers, storage tanks and practically any bulk container. Pays for itself by savings of time and product. Write for Bulletin.**

## THE CREAMERY PACKAGE MFG. COMPANY

**1243 W. Washington Blvd., Chicago 7, Ill.**

**Branches: Atlanta — Boston — Buffalo — Chicago — Dallas — Denver — Kansas City — Los Angeles — Minneapolis — Nashville — New York — Omaha Philadelphia — Portland, Ore. — Salt Lake City — San Francisco Seattle — Toledo — Waterloo, Iowa**

**CREAMERY PACKAGE MFG. CO. OF CANADA, LTD.**
**207 King St., West • Toronto, Ont., Canada**

**Your advertisement is being read in every State and in 25 Foreign Countries**

DIFCO

# LACTOBACILLUS ACIDOPHILUS

## *Isolation and Cultivation*

**Bacto-Trypsin Digest Agar** is an excellent culture medium for propagation of *Lactobacillus acidophilus.* The medium is prepared according to the formula of Cheplin. It is widely used for estimating the degree of intestinal implantation of *L. acidophilus* and is well suited for isolation of acidophilus strains and for carrying stock cultures.

**Bacto-Tomato Juice Agar** is prepared according to the formula of Kulp and White. The ability of this medium to support luxuriant and characteristic growth of *L. acidophilus* makes it particularly well adapted for use in establishing the number of viable organisms in acidophilus products. This medium is also used extensively in determining the degree of implantation of the organism.

**Bacto-Skim Milk** when prepared for use is an excellent medium for propagation of stock cultures of *Lactobacilli.* A 10 per cent solution of this product is equivalent to a high grade skim milk.

**Bacto-Peptonized Milk** contains degradation products of the proteins, albumins and globulins of milk. It supports rapid and luxuriant growth of the *Lactobacilli.*

*Specify "DIFCO"*

THE TRADE NAME OF THE PIONEERS
In the Research and Development of Bacto-Peptone and Dehydrated Culture Media

DIFCO LABORATORIES
INCORPORATED
DETROIT 1, MICHIGAN

Your advertisement is being read in every State and in 25 Foreign Countries